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Title: NC 03963 GENETIC INVESTIGATION OF NICOTIANA POPULATIONS

Project Leaders: E. A. Wernsman and D. F. Matzinger

I. Summary of Research:

A. Selection Response Based on Selfed Progenies

Selection based on progenies from single-seed descent ancestors is a common practice in naturally self-fertilizing species. Also, selfed progenies are candidates for selection for outbred performance. In the first case, the main question is how many generations of self-fertilization are needed or how does the response relate to the number of generations. In the second case, one question is how does response relate to the number of generations of selfing, and another question is how does the response relate to that for other forms of selection such as full-sib family selection.

A quantitative genetic model for arbitrary numbers of alleles and loci with additive, dominance, and additive by additive epistatic effects was employed. Utilizing descent measures and the genetic components, the selection response from selecting among self-fertilized relatives in generation g, where the last common ancestor was in generation t, was formulated. Permanent response, where g = infinity, led to considerable simplifications. The effect of the number of generations, t, of selfing before testing on the permanent response relative to the response from selecting among homozygotes was evaluated for a model with two loci, two alleles each. The result varied within a narrow range depending on gene frequencies and effects with a mean relative response of 0.68, 0.83, 0.92, 0.96, 0.98 for t = 1,2,3,4,5, respectively.

Selection response based on self-progeny evaluation was also formulated for the outbred performance of the offspring of randomly mated g's. On a per generation basis, the numerical evaluations gave about the same picture for t = 1 and t = 2 and less response for t greater than 2. If time is of little importance, the response generally increased with t, but there are parameter combinations of gene frequencies and effects for which the response is slightly negative.

These responses were compared to those from evaluating full-sib progenies from crossing pairs of self progenies, which give exactly the same result for t = 1 and t = 2 on a per generation basis. Self-progeny evaluations generally lead to more selection response than full-sib progenies except for those parameter combinations for which the response is slightly negative, and then it is very small for the full-sib progeny evaluation. Permanent response is slightly less than immediate

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response for inbred performance, and varies more with gene frequencies and effects when t is small. Permanent response for outbred performance can be much less than immediate response depending on the additive by additive variance.

B. ATPase Subunit 6 Gene Isolation (with Molly M. Bland)

The mitochondrial ATPase, one of the energy generating enzyme complexes of the inner membrane, consists of three components: F_0, F_1, and oligomycin sensitivity-conferring protein. These components function in the translocation of protons through the inner membrane and in the coupling of proton flux to ATP synthesis. A few of the subunits are encoded by the mitochondrial genome; the remaining components are nuclear encoded and imported into the organelle.

We have isolated and characterized the F_0-ATPase subunit 6 gene from tobacco mitochondria. The tobacco sequence exists as a single copy, is transcribed and contains an open reading frame capable of encoding a peptide of 395 amino acids. The first 130 amino acids of the tobacco putative polypeptide show limited homology with the N terminus predicted for the maize ATPase subunit 6. Although poorly conserved at the sequence level, the tobacco and maize amino termini are hydrophilic and have a high percentage of charged amino acids. This portion of the predicted peptide may represent a presequence that is common to the ATPase subunit 6 of plants.

Significant homology between tobacco and maize begins with amino acid 131, in a region that is highly conserved among fungal ATPase 6 subunits. In the remainder of the predicted protein, tobacco and maize share approximately 81% homology. A 41 bp sequence and a 175 bp conserved region found upstream from the tobacco atp6 coding region are homologous with sequence elements found in the 5' flanking regions of other plant mitochondrial genes and may be important for regulation and expression of the atp6 gene.

C. Rescue and Characterization of a Gametoclonal Variant Conferring Resistance to Potato Virus Y (with David Witherspoon and G. V. Gooding, Jr.)

In vitro anther culture has been shown to induce numerous genetic changes in haploid plants produced by this system. The genetic changes are permanent, and persist after numerous sexual reproductive cycles of the doubled haploid lines.

Potato virus Y (PVY) isolate NC 78 (strain NN) causes severe necrosis in young tobacco seedlings of all American flue-cured tobacco germplasm. At cool temperatures (24 C), flue-cured seedlings are usually killed within 21 days following inoculation.

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PVY-susceptible cultivar McNair 944 was subjected to in vitro anther culture and 5 cm. diameter haploid plants, along with diploid controls of the original parent, were inoculated with the PVY strain NN. Of 545 haploid plants inoculated, one plant survived, even though it possessed the virus. Leaf midvein explants of this plant were cultured in vitro, chromosome doubled plants were regenerated, and selfed seed were produced. Plants of the doubled haploid line, designated NC 602, and parental cultivar McNair 944 were tested for their reaction to the NN strain. NC 602 was resistant to the necrotic effects of this strain, and exhibited mild mosaic symptoms; all plants gave a uniform disease reaction. Conversely, all inoculated plants of McNair 944 developed severe necrotic symptoms and were killed.

NC 602 was crossed, reciprocally, with McNair 944, the F_1 and reciprocal F_1 were self pollinated to produce F_2 generations, and the F_1's backcrossed to both parents. Parents, F_1's, F_2's, and backcross progenies were inoculated with PVY NN. Data from the test confirmed that the doubled haploid line differed from parental cultivar McNair 944 by a single gene exhibiting additive effects (no dominance). Cytoplasmic and maternal effects on disease reaction were not observed.

NC 602 and McNair 944 were inoculated with a selected collection of 10 PVY isolates from the U.S. (five strains), Chile, Spain, South Africa, South Korea and Europe (two strains). A differential disease reaction between the two genotypes was found for three strains; U.S. strains NN and Vam-B, and the Spanish strain. The mutant gene appears to have value in the U.S. as a source of PVY resistance, since it provides protection against the necrotic reaction of the two severe PVY strains. These strains produce severe necrosis and death of McNair 944.

McNair 944 and NC 602 were grown in replicated yield tests at two locations and data were collected on six agronomic traits, as well as percentages of nicotine and reducing sugars in cured leaf lamina. NC 602 compared favorably with McNair 944 for all characters except leaf yield; it yielded approximately 14% less than McNair 944.

An F_2 population of NC 602 X McNair 944 was inoculated with PVY strain NN and 60 plants homozygous for the gene conferring PVY resistance were selected. Resistant selections were further inbred two generations by self pollination, and the 60 lines were evaluated for leaf yields and cured leaf quality. Significant genetic variation among lines for yielding ability was observed. It appears that it will be possible to recover the yield loss sustained in NC 602 while maintaining the complete PVY-resistance expressed in the gametoclonal variant.

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II. Graduate Students:

Bland, Molly M., Ph.D. Candidate, Genetics. Molecular genetics of tobacco.

Corbin, Thomas, C., Ph.D. Candidate, Crop Science. A comprehensive tobacco improvement program utilizing parthenogenic haploids.

Wilhite, Faye M., Ph.D. Candidate, Crop Science. Mass selection for quantitatively-inherited traits in tobacco haploid populations.

Witherspoon, William David, Jr., Ph.D. Candidate, Crop Science. Utilization of the haploid sporophyte as the selection unit in plant breeding.

Yung, Carl H., Ph.D. Candidate, Crop Science. DNA amplification in anther culture-derived haploids and its relationship to changes in tobacco metabolism.

III. Postdoctoral Fellows:

Bland, Molly M. Cloning of Nicotiana DNA.

IV. Publications:

Bland, M. M., C. S. Levings III, and D. F. Matzinger. 1987. The ATPase subunit 6 gene of tobacco mitochondria contains an unusual sequence. Curr. Genet. 12: 475-481.

Wernsman, E. A., and R. C. Rufty. 1987. Tobacco. p 669-698. In W. F. Fehr (ed.) Principles of Cultivar Development, Vol. 2. Macmillan Publishing Co. New York, NY.

V. Manuscripts in Review:

Matzinger, D. F., E. A. Wernsman, and W. W. Weeks. Restricted index selection for total alkaloids and yield in Nicotiana tabacum L.

Rufty, Rebeca C., E. A. Wernsman, and G. V. Gooding, Jr. Inheritance of resistance to tobacco etch virus in Nicotiana tabacum L. cultivar HA 307.

Witherspoon, W. D., Jr., and E. A. Wernsman. Feasibility of selection for quantitative traits among haploid sporophytes.

VI. Graduate Stud

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VII. Acknowledgmen

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VI. Graduate Student Theses Completed During Reporting Period:

Bland, Molly M. DNA sequence analysis of mitochondrial genes from Nicotiana tabacum. Ph.D. Thesis, 1987.

Corbin, Thomas C. Evaluation of a doubled haploid breeding procedure for simultaneous recurrent selection and inbred line development. Ph.D. Thesis, 1987.

VII. Acknowledgments:

This work was supported in part by the North Carolina Tobacco Foundation, Inc. and R. J. Reynolds Tobacco Company.

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Title: NC 05590 Characterization of cis-acting sequence determinants for the regulation of gene expression in plants.

Project Leader: Mark A. Conkling

I. Summary of Research:

This study was designed to examine the molecular mechanisms that regulate organ-specific gene expression in higher plants. These mechanisms are most easily conceptualized as trans-acting factors that interact with some cis-acting sequence determinants. An understanding of cis-acting sequences requires a correlation of structural and genetic data as they relate to gene expression. Therefore, systems allowing the in vitro purification and manipulation of genes and their subsequent reintroduction into cells (in vitro genetics) are required. Such experiments allow mutations to be targeted to putative regulatory regions and the effects of these mutations on expression to be determined.

Here we describe our progress towards the characterization of an organ-specific regulatory system in Nicotiana tabacum, tobacco. Tobacco was chosen as a model plant system since it is one of the plant species that exhibit totipotency, thus plants may be regenerated from single somatic cells. Additionally, efficient high capacity vector systems have been developed that integrate DNA into the genomes of most dicotyledonous plants (including tobacco). Finally, tobacco is a good genetic resource. These properties make tobacco an excellent experimental system for the study of cis-acting sequence determinants using the techniques of in vitro genetics. In addition, we describe the development of a novel genetic system allowing the cloning and isolation of cDNA's encoding trans-acting elements. This selection system may be used in conjunction with the cis-acting sequence determinants described above to characterize both cis-a and trans-acting determinants of organ-specific gene expression in tobacco.

Our progress for the past year is summarized below:

1. Characterization of genomic clones encoding the organ-specific cDNA clones.
2. Establishment of Agrobacterium-mediated transformation systems for promoter fusion experiments.
3. Development of genetic systems for the isolation of cDNA clones encoding trans-acting factors.

Using a novel mapping procedure, we have constructed preliminary restriction maps of both the root- and leaf-specific genomic clones. We have chosen to concentrate upon the genes encoding the root-specific cDNA clones. Fragments from these clones have been subcloned into either pUC or Bluescript vectors and detailed restriction maps prepared. The regions that hybridize to the cDNA clones have been defined and flanking regions have been used as probes in Northern analyses to help define the transcribed regions. To further define the transcribed regions of the genomic clones, all the clones

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II. Graduate Stud

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were digested with appropriate restriction enzymes and the fragments separated on agarose gels. These fragments were then Southern blotted to nitrocellulose filters and probed with reverse transcribed root mRNA. This probe was primed using random hexanucleotides such that the 3' termini of the mRNA molecules would not be over represented among the probe.

Before reintroduction into plant cells (transformation), the organ-specific genes must be "tagged" so that their expression may be distinguished form that of the endogenous genes. In reviewing potential reporter gene systems, we have chosen to use the chloramphenicol acetyltransferase, CAT, gene. Enzymatic activity of this gene's product is easily measured using thin layer chromatography. We will be transforming many organ-specific regulatory regions and various mutants into tobacco and thus felt it important to modify existing binary Agrobacterium vector systems to allow the rapid constructions of the tagged promoter. This vector allows the transcriptional and translational fusion of heterologous DNA to the CAT gene. It contains the neomycin phosphotransferase gene (NPT) for selection of transformed plant cells the T-DNA border sequences (B_L and B_R) necessary for plant transformation. Additionally, it contains a wide host range (WHR Ori) replicon to allow plasmid replication in Agrobacterium. We have produced transgenic plants with this vector and have begun promoter fusion experiments.

The analysis of trans-acting regulatory factors has lagged far behind that of the corresponding cis-acting elements. This is largely due to a lack of molecular biological "tools" with which to isolate such trans-acting factors. We have developed a selection system which employs the yeast Gal1 gene product. Gal1 encodes galactokinase and catalyzes the production of galactose-1-phosphate. If 2-deoxygalactose (2-DG) is present, expression of galactokinase generates a toxic compound (2-DG-1-P). Thus, cells expressing Gal1 are inhibited, while those not expressing Gal1 can grow. The Gal1 gene is under positive control of an Upstream Activating Sequence (UAS). We have found that binding a protein to the DNA sequences between the UAS and Gal1 genes inhibits the expression of the Gal1 gene. We can use this property to isolate cDNA's encoding DNA binding proteins. We constructed an integrating plasmid allowing suspected DNA-binding sequences (cis-acting regulatory sequences) to be easily cloned between the UAS and Gal1. A cDNA library cloned into a multi-copy yeast expression vector may then be transformed into these cells and the transformants subjected to selection on 2-DG. Survivors are likely to be cells carrying cDNA sequences encoding proteins that interact with specific DNA sequences located between the UAS and Gal1, i.e. trans-acting factors.

II. Graduate Students:

Yuri Yamamoto (Genetics)

Judy Jakobek (Botany)

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III. Postdoctoral Fellows:

Dr. Danian Huang - Visiting scientist from the China National Rice Institute, Hangzhou, Peoples' Republic of China. December 20, 1987 - May 15, 1988.

VII. Papers Presented at Professional Meeting:

Organ-specific gene expression in tobacco. Gordon Conference on Plant Molecular Biology

Biotechnology is changing agriculture. Philip Morris Young Leadership Conference.

IX. Acknowledgements:

I wish to thank Ms. Carolyn Faucette and Dr. Georgia Acedo for their excellent technical assistance. I also wish to acknowledge Dr. Judy Thomas and the staff of the Southeastern Plant Environmental Laboratories for their help in growing tissue under controlled environmental conditions.

This work was supported by the North Carolina Tobacco Foundation and the North Carolina Biotechnology Center.

Title:

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I. Summar

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Title: NC03896 Cell Culture Techniques for the Development of Disease Resistance in Tobacco

Project Leader: Margaret E. Daub

I. Summary of Research:

This project has three broad objectives:

1. To investigate the potential of somaclonal variation for generating novel, disease-resistant genotypes.

2. To develop protoplast fusion techniques for transferring naturally-occurring resistance genes from wild Nicotiana species into tobacco cultivars.

3. To investigate the molecular interactions in one host-pathogen system (the tobacco/Cercospora nicotianae system) in order to identify genes which may be useful in the engineering of disease-resistance plants.

A. Use of somaclonal variation for generating disease-resistant tobacco. The purpose of this work is to investigate the usefulness of somaclonal variation (variation induced in tissue culture) for developing high quality tobacco cultivars with increased levels of disease resistance. We have generated over 1000 somaclones from the flue-cured tobacco cultivars NC2326, Coker 319, Speight G-28, and K399. We have recently completed three years of field evaluation of the NC2326 and Coker 319 somaclones. Variation was noted in yield, leaf chemistry, and black shank and Granville wilt resistance. We did not detect variation in root knot nematode (M. incognita) or tobacco mosaic virus resistance. Variation which was seen was slight and often not desirable. Many lines showed greater susceptibility to black shank, for example. Better results have been obtained with the Speight G-28 and K399 somaclones. Again, variation in disease resistance often tended to be in the direction of greater susceptibility. However, one K-399 somaclone was identified whose progeny showed no losses in two Granville wilt nurseries in 1987. Also, Speight G-28 somaclones appear to show significant variation in resistance to Meloidogyne arenaria. Studies with these somaclones are continuing.

B. Transfer of resistance genes from wild Nicotiana species to Nicotiana tabacum by protoplast fusion. The present goal of this work is to devise methods for selecting hybrid cell lines following fusion of protoplasts. With present protocols, maximum hybrid fusion frequencies are around 5-10%, thus necessitating some means of separating hybrids from the unfused parental protoplasts. The selection scheme used previously (the

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albino marker Su/Su) was determined to be unsuitable for hybrids between highly incompatible species due to its high reversion rate, poor survival of chlorophyll-deficient plants, and the inability to utilize electrofusion methods when using this system. Our current selection scheme involves transforming the parent plants for resistance to antibiotics and using antibiotic selection to select hybrids. We obtained two Agrobacterium tumefaciens transformation vectors from Ciba-Geigy Corporation. These vectors transform plants for resistance to hygromycin and kanamycin. We have obtained kanamycin and hygromycin-resistant transformants of NC2326, Burley 21, and an oriental tobacco cultivar (Nevrocop A-24). We have been unable to transform Nicotiana repanda. N. repanda tissues become necrotic when treated with these vectors, and we suspect that N. repanda may be expressing a hypersensitive-type resistance to the vector. To get around this problem, we are currently working to transform N. sylvestris, and intend to transfer the antibiotic-resistance genes into N. repanda by conventional crosses. We are also transforming N. debneyi and N. alata. We have worked out conditions for selecting hybrids. Best survival of resistant cell lines occurs if selection is applied approximately 6 weeks after protoplast isolation. Cell lines are selected on medium containing 100 ug/ml kanamycin and 40 ug/ml hygromycin.

C. The Phytotoxin Cercosporin produced by Cercospora species. Two areas of research are currently being pursued; i) studies on the genetic and biochemical basis of resistance to cercosporin, and ii) studies on the regulation of toxin synthesis by the fungus. We have determined that the primary mechanism of resistance of Cercospora species to cercosporin is due to the presence of some factor in the Cercospora cell wall which protects the cell against oxidative damage by cercosporin-generated singlet oxygen. We are attempting to isolate this factor from cell wall preparations. We are also attempting to identify the genes responsible for toxin resistance. We have constructed a genomic library of C. nicotianae DNA in the fungal transformation vector pSV50. The DNA vector library will be used to transform Neurospora crassa, which is sensitive to cercosporin. We hope to isolate cercosporin-resistance genes by selecting for cercosporin-resistant N. crassa transformants. We have also been studying regulation of cercosporin synthesis by the fungus. We have completed an extensive study of nutritional and environmental factors that regulate cercosporin production. We have identified three environmental triggers that can rapidly turn on or shut off toxin synthesis in several isolates. We are using 2-D gel electrophoresis to analyze differences in proteins present when isolates are grown under cercosporin-producing or non-producing conditions. If protein differences are identified, we can use antibodies to these proteins to study toxin regulation. The antibodies can also be used to isolate the

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IV. Publications:

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corresponding genes from a cDNA library cloned in an expression vector. We are hoping to identify genes which negatively regulate toxin production. If genes are identified that control toxin resistance and/or toxin regulation, we will attempt to express these genes in plants with the eventual goal of obtaining disease-resistant plants.

II. A. Graduate Students:

Cynthia Cooperman, Ph.D. candidate in Plant Pathology. Thesis Topic: "Cloning and characterization of genes coding for cercosporin resistance in Cercospora nicotianae."

II. B. Special Students:

Angela Frazier, Microbiology Major
Honors Research project on DNA isolation from Cercospora species.

Jeffrey A. Rollins, R. J. Reynolds apprenticeship program. Project: Protoplast fusion in tobacco.

III. A. Postdoctoral Fellow:

Dr. Anne E. Jenns. Research Area: Regulation of cercosporin production.

III. B. Visiting Scientist:

Dr. Velichka Nikolaeva, visiting scientist from Bulgaria. Research Area: Selection of tobacco cell cultures for resistance to tabtoxin.

IV. Publications:

Daub, M. E. 1987. The fungal photosensitizer cercosporin and its role in plant disease. pp. 271-280 in J. R. Heitz and K. R. Downum, eds. Light-Activated-Pesticides. American Chemical Society, Washington, D. C.

Daub, M. E. 1987. Resistance of fungi to the photosensitizing toxin cercosporin. Phytopathology 77:1515-1520.

V. Manuscripts Accepted for Publication:

Gwinn, K. D. and M. E. Daub. 1988. Regenerating protoplasts from Cercospora and Neurospora differ in their response to cercosporin. Phytopathology 78 (In Press).

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Hartman, P. E., W. J. Dixon, T. A. Dahl, and M. E. Daub. 1988. Multiple modes of photodynamic action by cercosporin. Photochem. Photobiol. 47 (In Press).

Fore, S. A., M. E. Daub, and M. K. Beute. Phytotoxic substances produced by some isolates of Cercospora arachidicola are not cercosporin. Phytopathology (accepted).

VI. Manuscripts in Review:

Daub, M. E., and G. A. Payne. Role of carotenoids in resistance of fungi to cercosporin. (Submitted to Phytopathology).

Jenns, A. E., M. E. Daub, and S. M. Reed. Limitation of the Su albino marker system in fusions between Nicotiana repanda and Nicotiana tabacum. (In departmental review).

Jenns, A. E., M. E. Daub, and R. G. Upchurch. Regulation of cercosporin production by Cercospora spp. (In departmental review).

VII. Papers Presented at Professional Meetings:

Jenns, A. E., M. E. Daub, and R. G. Upchurch. 1987. Regulation of cercosporin production by growth media components. Phytopathology 77:1701. (Abstract).

Fore, S. A., M. E. Daub, and M. K. Beute. 1987. Phytotoxic substances produced by certain isolates of Cercospora arachidicola are not cercosporin. Phytopathology 77:1719. (Abstract).

IX. Acknowledgements:

The excellent technical assistance of Mr. D. Wayne Pollard is greatly appreciated. This work has been supported by funds from R. J. Reynolds Corporation, The North Carolina Tobacco Foundation, and The North Carolina Biotechnology Center.

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Project Leader: G. W

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Title: NC 03794 Virus Diseases of Solanaceous Crops

Project Leader: G. V. Gooding, Jr.

I. Summary of Research:

The most important viruses of tobacco in North Carolina are transmitted by aphids. Virus-resistant flue-cured tobacco varieties are not available for the control of these viruses. Field studies with tobacco in the 1970's in North Carolina demonstrated that the use of insecticides did not reduce virus incidence. Therefore, it has been recommended that insecticides not be used in an attempt to control these viruses on tobacco. The use of insecticides for this purpose would be a waste of money and add additional residues to the crop. In the early 1980's, it was reported that with burley tobacco in Kentucky, some reduction in virus incidence occurred in some field plots in some years by using insecticides to control aphid vectors. The effect of insecticides on virus incidence in flue-cured tobacco was tested again from 1985-1987 in North Carolina to determine if conditions may have changed. There was no difference in virus incidence in plots treated with insecticides and those not treated. Aphid control in the treated plots was complete while high populations of aphids developed in untreated plots. Based on these data, insecticides should not be used in an attempt to control aphid-transmitted viruses on flue-cured tobacco in North Carolina. Control is based on the elimination of sources of the virus.

Cooperative research was continued with tobacco breeders to identify and incorporate resistance to tobacco viruses in agronomically acceptable cultivars. A three-year field study was completed in which burley tobacco cultivars were evaluated for their tolerance to tobacco etch and tobacco vein mottling viruses. Some cultivars such as TN86 and Ky14 were highly tolerant to both viruses.

A study was initiated in cooperation with Drs. Pi-Yu and Jeng Sheng Huang to investigate the physiological and biochemical factors associated with differential reactions elicited by different strains of potato virus Y on different tobacco germplasm. Three potyviruses, tobacco vein mottling virus (TVMV), tobacco etch virus (TEV), and potato virus Y (PVY), cause significant losses on tobacco in North Carolina. In contrast to TVMV and TEV, PVY consists of a highly divergent population of strains that induce symptoms on tobacco ranging from only a mild mottling to plant death. This multiplicity of PVY strains complicates breeding efforts to develop resistant cultivars and other control approaches. An understanding of the biochemical basis for these differential reactions would support the breeding program as well as other approaches to control. In a preliminary study, NC2326 and NC95 were inoculated with the MN strain of PVY, sampled, and tissue observed at different time intervals after inoculations for different morphological changes. Dr. Pi-Yu Huang found that one of the earliest differential responses is the disintegration of the chloroplast membrane in NC95 but not NC2326. This observation targets at least one factor resulting in death of NC95 plants infected with the MN

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strain of PVY. The next phase of the study will be to examine the biochemical basis for chloroplast membrane disintegration.

II. Publications:

Gooding, G. V., Jr., and R. C. Rufty. 1987. Distribution, incidence, and strains of viruses in burley tobacco in North Carolina. Plant Disease 71:38-40.

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Averre, C. W., and G. V. Gooding, Jr. 1987. Potato virus Y. Plant Path. Inf. Note 1/87/500. (Revised)

Rufty, R. C., E. A. Wernsman, and G. V. Gooding, Jr. 1987. Evaluation of tobacco haploids and double-haploids for resistance to tobacco mosaic virus, Meloidogyne incognita, and Pseudomonas syringae pv. tabaci using detached leaves. Phytopathlogy 77:60-62.

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Gooding, G. V., Jr., and P. B. Shoemaker. The tobacco vein mottling and tobacco etch diseases of burley tobacco. Plant Path. Inf. Note.

Lampert, E. P., H. Amy Smith, and G. V. Gooding, Jr. Transmission of potato virus Y and tobacco etch virus by red and green color forms of the green peach aphid. J. Econ. Entomol.

Lampert, E. P., and G. V. Gooding, Jr. Suppression of green peach aphids (Homoptera: Aphididae) on flue-cured tobacco and its effects on the incidence of potato virus Y. J. Econ. Entomol.

Rufty, R. C., E. A. Wernsman, and G. V. Gooding, Jr. Inheritance of resistance to tobacco etch virus in Nicotiana tabacum L. cultivar Havana 307. Plant Disease.

Rufty, R. C., R. D. Miller, and G. V. Gooding, Jr. Evaluation of burley tobacco genotypes for tolerance to tobacco etch and tobacco vein mottling viruses. Plant Disease.

IV. Acknowledgement.

This work was supported in part by the North Carolina Tobacco Foundation, Inc. Appreciation is extended to J. B. Young for excellent technical assistance.

Title: NC 3773 Biol

Project Leader: H.

I. Summary of Rese

Black Root Rot effect of host resis Thielaviopsis basico was continued in 198 Dr. Paul Shoemaker. strides have been ma of T. basicola resis The Nicotiana tabacu protection at best a most resistant culti resistance should nc tobacco culture. Cu is planted to cultiv fumigation reduces b Populations of T. ba fumigation. Soil co greatly affect effic populations have ran populations of T. ba the growing season i general never equal susceptible or low r developed on roots c resistance. Therefo tobacco culture in debneyi resistance a rotation (with P. B.

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Title: NC 3773 Biology and Epidemiology of Tobacco Root Diseases

Project Leader: H. D. Shew

I. Summary of Research:

Black Root Rot - The on-farm testing program to determine the effect of host resistance and soil fumigation on populations of Thielaviopsis basicola and the yield and quality of burley tobacco was continued in 1987. This work is being done in cooperation with Dr. Paul Shoemaker. Over the last two growing seasons significant strides have been made as to the effectiveness of different sources of T. basicola resistance in reducing losses to the black root rot. The Nicotiana tabacum source of resistance provides low to moderate protection at best and allows build up of the pathogen even in the most resistant cultivars. Thus, cultivars with this source of resistance should not be recommended in fields in continuous tobacco culture. Currently over 80% of our burley tobacco acreage is planted to cultivars containing N. tabacum resistance. Soil fumigation reduces but does not eliminate the pathogen from soil. Populations of T. basicola are not always greatly reduced by soil fumigation. Soil conditions at the time of fumigation probably greatly affect efficacy of the fumigants. Reductions in populations have ranged from 17 to 93% following fumigation. Soil populations of T. basicola may continue to be affected throughout the growing season if very high rates of fumigant are used, and in general never equal populations from untreated rows even if a susceptible or low resistant variety is planted. No disease has developed on roots of cultivars containing the N. debneyi source of resistance. Therefore, if a grower must practice continuous tobacco culture in a field, he can use the cultivars containing N. debneyi resistance as a non-host crop and essentially practice crop rotation (with P. B. Shoemaker).

The presence and populations of the black root rot pathogen Thielaviopsis basicola and occurrence of black root rot on burley tobacco were determined in a random survey of 80 burley tobacco fields in the burley growing region in western North Carolina. The pathogen was detected in 58% of the survey fields at a mean population density of 40 CFU/g soil. Black root rot was observed in 70% of the infested fields. Disease rating was positively correlated with inoculum density of T. basicola. For example, moderate or severe disease symptoms occurred only at inoculum densities greater than 60 CFU/g soil. Disease ratings on moderately resistant cultivars were significantly lower than on cultivars with low resistance to black root rot. No disease was detected on the very highly resistant cultivar TN 86 in any field. Disease ratings on cultivars with low resistance to black root rot were highly correlated with exchangeable soil Ca, Mg, K, CEC and

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base saturation, but disease ratings on moderately resistant cultivars were not. Pathogen populations and disease severity were significantly lower in fields in which rotation was practiced. The results of the survey indicate that T. basicola is widely distributed in the burley tobacco growing region of western North Carolina and that both pathogen inoculum density and soil pH-related chemical variables are good indicators for predicting favorable conditions for black root rot development.

A phytotron experiment was completed to test if isolate virulence and soil chemical factors explain differences in disease severity found among the survey fields. An initial factorial experiment was completed, in which isolates of T. basicola collected during the survey were tested for differences in virulence on 3 cultivars, with low, moderate and high black root rot resistance, in 3 field soils, with pH values of 4.5, 6.0 and 6.7. The soils were fumigated before use and infested with T. basicola to the same inoculum density. Burley tobacco plants were rated for symptoms of black root rot after 3 wks. Disease severity was significantly different among isolates, indicating that isolates differ in general virulence. Cultivar x isolate interactions were also detected. Disease severity was strongly affected by soil type. For example, mean disease ratings were 1%, 24% and 78%, in an acid, moderately acid and neutral soil, respectively. This pattern was consistent for all cultivar and isolate combinations. T. basicola was detected in all soils at the end of the experiment, suggesting that the infection process and not inoculum survival was affected by soil factors.

Black Shank - Investigations into components of resistance of several varieties of tobacco to Phytophthora parasitica var. nicotianae were initiated. Perspex root observation boxes were designed and built by the agricultural engineering workshop. A procedure for growing field size transplants in microwaved soil was developed. Exposure of soil to seven minutes and thirty seconds of microwaves was established as an acceptable method of pasteurizing soil. A system for inoculating motile zoospores onto essentially undisturbed roots growing against the clear perspex face of the root observation box also was developed. One-mm-diameter holes are drilled into the root observation box just in front of the root tip and zoospores are added as the root grows by the hole. The same one-mm-holes will be used to collect zoospores produced on root lesions by inserting micropippet tubes containing 0.025M ethanol as a bait. Several methods were evaluated for determining root length production. The Apple microcomputer based Dapple systems were found to lack the definition required to reliably estimate root lengths. It has been decided that a digitizing tablet based system will allow the most accurate measurement of root and lesion lengths.

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A standard curve was generated using a new immuncassay technique to quantify zoospore number in water and soil solution. Sample preparation techniques and immunoassay technique protocol were optimized. The resulting double sandwich ELISA technique was sensitive to as little as two zoospores per 100 uml.

Field studies were conducted to observe natural infections of roots of a susceptible and a resistant tobacco cultivar. A ridomil drench was used to reduce inoculum in one treatment. Plants were sampled destructively at three day intervals after transplanting and root production was estimated. Observations (lesions and pathogen recovery on selective media) suggest that similar levels of infection occurs in all varieties regardless of their level of resistance and that resistance in the field is the result of other factors.

Studies were performed to determine the effect of soluble aluminum (Al) on the growth and motility of P. p. var. nicotianae. Aluminum nitrate concentrations greater than 0.4 meq/100g (36 ppm) in V-8 juice agar significantly inhibited mycelial growth of P. p. nicotianae (isolates 411 and 455). Similar levels of calcium nitrate stimulated mycelial growth. Raising medium pH from 4.5 to 5.0 or 6.0 significantly increased growth in Al treatments (ionic species of Al present is in transition in this pH range). Aluminum levels of greater than 0.01 meq/100g (0.9 ppm) in deionized water and 10% Hoaglands solution caused zoospores to encyst within 5 minutes whereas solutions with no Al or similar levels of Ca present allowed zoospores to remain motile for more than 5 hours. These observations may help explain why this disease never becomes a serious problem in some fields whereas in other fields the disease becomes very severe.

Studies on inoculum production by P. p. nicotianae on cultivars of flue-cured tobacco was continued in microplot tests. Four cultivars from each of three resistance classes (low, moderate, and high) were planted in microplots infested with the pathogen. Days to symptom expression and populations of P. p. nicotianae in the root zones of plants four weeks post symptom expression were determined. Symptom development was delayed as the level of resistance increased. However, populations of P. p. nicotianae were not related to the level of resistance expressed under field conditions. These results suggest that there are multiple mechanisms of resistance to P. p. nicotianae in tobacco.

II. Graduate Students:

DeLuca, T. H. Ph.D. Characterization of Suppression of Phytophthora parasitica var. nicotianae in Select North Carolina Soils.

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Jones, K. J. Ph.D. Investigation into the dynamics of root infection by Phytophthora parasitica var. nicotianae.

Meyer, J. R. Ph.D. The effect of edaphic factors on the incidence and severity of black root rot of tobacco, caused by Thielaviopsis basicola.

IV. Publications:

Shew, H. D. 1987. Effect of host resistance on spread of Phytophthora parasitica var. nicotianae and subsequent development of tobacco black shank under field conditions. Phytopathology 77:1090-1093.

VI. Manuscripts in review:

Meyer, J. R., Shew, H. D., and Shoemaker, P. B. 1988. Populations of Thielaviopsis basicola and the occurrence of black root rot on burley tobacco in ten western North Carolina counties. Plant Disease 72:000-000.

IX. Acknowledgements:

I gratefully acknowledge the excellent technical assistance of Derald Glover.

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I. Summary of

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BIOLOGICAL CONTROL OF CERTAIN FOLIAR PLANT DISEASES AND NEMATODES IN TOBACCO

Harvey W. Spurr, Jr.

Research Leader-Laboratory Director, Research Plant Pathologist,
United States Department of Agriculture, Agricultural Research Service;
Professor of Plant Pathology, N.C. State University
State 05615

I. Summary of Research:

This project is concerned with disease control mechanisms and their application to achieve plant disease control in a practical and economical manner. The emphasis is on new approaches and new techniques for understanding and efficiently controlling diseases.

Research during 1987 includes the following results and activities.

Tobacco Blue Mold: A series of biochemical interactions between the tobacco leaf and fungal spores appear necessary to the development of a functional relationship between host and pathogen. Our first objective is to identify the biochemical components necessary for the specific interaction of Peronospora tabacina with tobacco resulting in colonization by the fungus. The ability of spores to adhere to the surface of a host is a key step in the initiation of disease. P. tabacina spores adhere tenaciously to tobacco leaves indicating to us that adhesion is involved in specificity of the pathogen for tobacco. P. tabacina spores were placed on glass and on tobacco, tomato, petunia, and bean leaf discs and tested for adhesion. The spores adhered to all surfaces. Treating the spores with a protease resulted in a significant decrease in binding to tobacco leaf surfaces. Therefore, a protein may be involved in adhesion of these fungal spores. We conclude that adhesion is a general property of P. tabacina spores and not related to host recognition. Our second objective is to determine how high humidity induces sporulation of P. tabacina in leaves. Experiments to determine the biochemical pathway activated by high humidity are being initiated. (Cooperator: Mary L. Menetrez)

The genetic composition of the fungal pathogen, P. tabacina, determines its response to the environment. With this obligate pathogen, genetic composition is the principal determinant of survival. Two studies are directed at determining this genetic composition and how it functions: 1) A world collection of tobacco tissue infected with P. tabacina is being studied to determine oospore morphology and distribution and thusly, the function of the sexual cycle. 2) A world collection of isolates of P. tabacina is being assembled at the Plant Disease Laboratory, Ft. Detrick, MD. This is the only approved containment facility in the U. S. and the personnel have much experience with downy mildews. Once the collection is assembled pathogen variability will be studied. (Cooperators: C. E. Main, M. R. Bonde)

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Biological Control of Plant Pathogens: Environmental conditions favorable to black Sigatoka of banana (caused by Mycosphaerella fijiensis var. difformis) were investigated. Environment (daily temperature, hourly rainfall, daily relative humidity, hours cloudiness/day, daily evaporation) and disease (ascospore counts, severity) were measured on a banana plantation in the Atlantic zone of Costa Rica. Daily spore count had the highest correlations with hours cloudiness (r=0.64), hours rainfall (r=0.61) and evaporation (r=0.56). The correlation between hourly spore counts and hourly rainfall was 0.38 for measurements from the same hour, but was near zero between spore count and rainfall in preceding hours. Disease severity correlated best to maximum temperature (r=0.35) and hours rainfall (r=0.33). Models are being developed incorporating these data to improve selection and timing of fungicides and to develop biological control strategies. (Cooperator: W. Thal)

Field tests during the summer of 1987 in two locations in North Carolina and one in Virginia afforded an opportunity to evaluate production, formulation, application and disease evaluation systems for biological control of peanut leafspot disease. These data are being analyzed. (Cooperator: V. J. Elliott) Two bacterial formulations were tested for control of tobacco blue mold in Puerto Rico. (Cooperator: C. E. Main) A field test at Oxford, NC evaluated several methods for introducing bacterial antagonists to tobacco root systems for the control of Granville wilt.

III. Postdoctoral Fellows:

Dr. Vernon J. Elliott, ARS-USDA Visiting Scientist, Biological Control of Foliar Pathogens. Pilot Test Program.

Dr. Mary L. Menetrez, Postdoctoral Fellow, Host Specificity and Sporulation of Peronospora tabacina, the Blue Mold Pathogen, Sponsor: R. J. Reynolds Tobacco.

Dr. Wayne M. Thal, Research Affiliate USDA-ARS, Biological Control of Leafspot Diseases, Sponsor: Del Monte Corporation.

IV. Publications:

Knudsen, G. R. and Spurr, H. W., Jr. 1987. Field persistance and efficacy of five bacterial preparations for control of peanut leafspot. Plant Disease 71:442-445.

Trigiano, R. N. and Spurr, H. W., Jr. 1987. The development of the multinucleate condition of Peronospora tabacina sporangia. Mycologia 79:353-357.

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Knudsen, G. R., Spurr, H. W., Jr. and Johnson, C. S. 1987. A computer simulation model for Cercospora Leafspot of Peanut. Phytopathology: 71:1118-1121.

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Thal, W., Arroyo, T., and Spurr, H. W., Jr. 1987. Relationship between environment and black Sigatoka of banana. Phytopathology 77:1730. (Abstr.)

Menetrez, M. L., Spurr, H. W., Jr., Danehower, D. A., and Lawson, D. R. 1987. Toxicity of leaf surface chemicals to Peronospora tabacina. 41st Tobacco Chemists Research Conference, p. 44. (Abstr.).

V. Manuscripts Accepted for Publication:

Knudsen, G. R. and Spurr, H. W., Jr. 1987. Management of bacterial populations for foliar disease biocontrol. in: Mukerji, K. G. and Garg, K. L., eds. Biocontrol of Plant Diseases. CRC Press: Boca Raton, FL. (in press).

Knudsen, G. R., Johnson, C. S. and Spurr, H. W., Jr. 1987. Use of a simulation model to explore fungicide strategies for control of Cercospora leafspot of peanut. Peanut Sci. 14: (in press).

Menetrez, M. L. and Spurr, H. W., Jr. 1988. Specificity of Peronospora tabacina. in: W. E. McKeen ed., Blue Mold of Tobacco. APS Books, St. Paul, MN. (in press).

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Lawson, D. R., Danehower, D. A., Menetrez, M., and Spurr, H. W., Jr. 1987. Allelochemical properties of Nicotiana tabacum leaf surface compounds. Agric. and Food Chem. Symposium, ACS. (Abstr.) (in press).

VI. Manuscripts in Review:

Spurr, H. W., Jr. and Menetrez. M. L. 1988. Pathogen variation and specificity. International Symposium on Blue Mold Disease of Tobacco (in review).

Menetrez, M. L. and Spurr, H. W., Jr. 1988. Leaf surface phenomenon and specificity of infection. International Symposium on the Blue Mold Disease of Tobacco (in review).

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VII. Papers Presented at Professional Meetings:

Menetrez, M. L., Spurr, H. W., Jr., Danehower, D. A., and Lawson, D. R. 1987. Toxicity of leaf surface chemicals to Peronospora tabacina. 41st Tobacco Chemists Research Conference, Greensboro, NC. Oct. 4-7 ,1987.

Spurr, H. W., Jr. and Thal, W. 1987. Field evaluation of bacterial antagonists for peanut leafspot control. 19th Annual Meeting American Peanut Research Soc., Inc. Orlando, FL.

Lawson, D. R., Danehower, D. A., Menetrez, M., and Spurr, H. W., Jr. 1987. Allelochemical properties of Nicotiana tabacum leaf surface compounds. Agric. and Food Chem. Symposium, ACS, Washington DC.

Spurr, H. W., Jr. and Menetrez, M. L. 1988. Pathogen variation and specificity. International Symposium on the Blue Mold Disease of Tobacco, Raleigh, NC, February 14-17, 1988.

Menetrez, M. L. and Spurr, H. W., Jr. Leaf surface phenomenon and specificity of infection. International Symposium on the Blue Mold Disease of Tobacco, Raleigh, NC, February 14-17, 1988.

IX. Acknowledgements:

I thank Catherine Currin, Linda D. Daniel, and Henry "Buddy" Quick for technical assistance.

TITLE: NC 0379

PROJECT LEADER:

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TITLE: NC 03791 Epidemiology and Control of Foliar Disease of Tobacco

PROJECT LEADER: C. E. Main

I. Summary of Research:

This project has the following objectives:

A. Monitor occurrence, distribution, and importance of foliar diseases of tobacco in North Carolina.

B. Determine the source(s) of primary inoculum of Peronospora tabacina.

C. Develop improved control tactics for foliar diseases using fungicides, resistant cultivars, and cultural practices.

D. Monitor the presence of ecotypes, strains and races of foliar pathogens.

E. Relate development and spread of foliar diseases to weather and climate.

F. Assess the impact of foliar diseases on yield and value.

G. Establish cooperative efforts on foliar diseases with state, federal and international research centers.

Studies on the Blue Mold Disease of Tobacco

Blue mold, caused by the obligate fungus pathogen Peronospora tabacina, continues to be a serious disease threat to the tobacco industry in the United States and Latin America. Macroscale, explosive epidemics generally occur when the growing tobacco crop, the pathogen, and conducive weather conditions co-exist. During 1987-88, scientists at North Carolina State University and the USDA Tobacco Research Laboratory at Oxford, NC, studied the biology, epidemiology, biometeorology, development of resistant cultivars, fungicidal control strategy, and fungicide residues using both flue-cured and burley tobaccos.

The objectives of the blue mold project are comprehensive and broad based involving: (1) applied approaches to breeding resistant varieties, formulating fungicidal control strategies and educating the tobacco growers, (2) basic investigations into the genetics and physiology of host resistance, (3) ecologic and genetic variability within the pathogen population, (4) biometeorological aspects of inoculum movement and disease spread, and (5) development of an education and contingency control program in the event a metalaxyl resistant strain of P. tabacina should be introduced into the United States. This report describes on-going

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research on this important disease problem.

Blue mold occurred in July in the Burley region of NC but spread was limited. Therefore, the North Carolina tests were once again used to evaluate the type, yield and quality of blue mold resistant breeding lines and to conduct fungicide residue analyses on the cured-leaf. Eighty percent of the research experiments at Gurabo, Puerto Rico were destroyed by two severe floods. Some data on the fungicide strategy tests were salvaged. Valuable disease evaluations were obtained in the winter nursery tests on the east coast of Mexico. Results are not yet available from experiments located on the west coast of Mexico.

BREEDING AND GENETICS (With Rebeca Rufty and E. A. Wernsman)

The objectives of the plant breeding studies are to: (1) evaluate blue mold resistant flue-cured and burley germplasms for improved agronomic traits, chemical composition and performance, (2) determine the usability of the germplasm as a source of resistance, and (3) identify and characterize additional and/or new sources of blue mold resistance which may be utilized in the development of breeding lines. The long-term goal is to develop tobacco genotypes possessing multigenic resistance mechanisms thereby increasing the quantitative level of resistance. Such quantitative resistance may be more stable to pathogen variation and less vulnerable to population shifts in P. tabacina.

Thirty-one burley tobacco breeding lines in the F5 generation were evaluated in 1987-88 for blue mold (P. tabacina) resistance at three locations: Gurabo, Puerto Rico, and San Andres Tuxtla and Papantla, Mexico. Breeding lines, derived from crosses of resistant cultivar Ovens 62 x susceptible cultivars Ky 17 and Ky 15, were planted at each location in a randomized complete block design with three replications. Disease evaluations for relative blue mold reactions were performed in December 1987 and January 1988. Excellent results were obtained at the two Mexican sites where a Ridomil-resistant strain of P. tabacina prevails. All the experimental material at Gurabo, Puerto Rico was lost due to the presence of black shank (Phytophthora parasitica var. nicotianae) and to two severe floods.

Susceptible cultivars Ky 15, Tn 86 and Ky 17 exhibited disease levels ranging from 20 to 53% leaf area damaged. Resistant cultivars Ovens 62 exhibited only 7% leaf area damaged. Twelve highly resistant burley breeding lines were selected with disease reactions equal to or lower than those of the resistant Ovens 62. The resistant lines will be self-pollinated and advanced to the F6 generation for further evaluation. These lines will also be used as resistant parents in a second cycle of breeding.

Therefore, genetically-stable burley tobacco lines have been identified which offer a high degree of resistance to tobacco blue mold. These lines appear to be resistant to both Ridomil-sensitive and Ridomil-resistant strains of the blue mold fungus. In 1986-87, thirty-five flue-

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cured and burley lines with resistance to blue mold, plus appropriate susceptible checks, were planted at two locations in Mexico (Papantla & San Andres in the state of Veracruz on the east coast) where the metalaxyl resistant strain is present. Blue mold reaction and entry resistance rankings were identical to those obtained in Puerto Rico since 1983. Therefore, our resistant germplasm is expected to provide protection in the eventuality that metalaxyl resistant strains of P. tabacina are introduced into the United States.

The N.C. Breeders Release Board recommended the release of NC-BMR-42 and NC-BMR-90 in 1987. Because of adequate resistance to blue mold and acceptable agronomic characteristics, NC-BMR-42 represents the best germplasm source available in the United States for development of flue-cured resistant cultivars. NC-BMR-90, with 50% burley tobacco germplasm, may be used by burley breeders. Presently there are no acceptable burley cultivars with blue mold resistance.

A second breeding cycle for the development of blue mold-resistant flue-cured and burley breeding lines has been initiated using genetic materials shown to be resistant to the disease during 1985-86 evaluation experiments in Puerto Rico. Flue-cured breeding lines used as sources of disease resistance included NC BMR 42 (DH 42) and NC BMR 62 (DH 62), doubled haploid lines originating from the cross Ovens 62 x McNair 944; and D-2, a line with a Chemical Mutant resistance mechanism derived from the cross Chemical Mutant x Speight G-28. Second cycle burley breeding lines were developed from crosses of Ky 17 with NC BMR 90, a half-burley doubled haploid line from the cross Ovens 62 x Ky 17, and breeding lines possessing the burley phenotype and the Chemical Mutant resistance mechanism.

F1 hybrids of blue mold-resistant and susceptible parents were crossed with N. africana and haploid plants identified in surviving seedlings from the interspecific crosses. Midvein explants from leaves of haploid plants were subjected to in vitro tissue culture, and chromosome-doubled. Regenerated plants were identified and self pollinated. Many of the lines were characterized for their heterozygous for these genes. Approximately 80% of the lines were planted in Puerto Rico in 1987, but the tests were lost in a flood. Remnant seed of the lines shown in the following table are available for further tests.

An array of genetic materials has been developed to discern whether it is possible to combine in a single genotype, blue mold resistance mechanisms originating from N. velutina and N. goodspeedii. NC BMR 42, with the N. velutina resistance mechanism, was hybridized with ZA 692, a highly disease-resistant line with a N. goodspeedii mechanism. Fifty random F4 lines were produced to determine if any disease susceptible families were produced in the array. Uniformity of resistance among the lines would be interpreted as evidence that the genomic site of insertion of genes for resistance is homologous in the two breeding lines. The presence of disease susceptible lines in the array would suggest that genes for resistance in the two parents are different and could be

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combined in a single genotype.

FUNGICIDE EVALUATION AND STRATEGY (With P. B. Shoemaker)

Fungicide tests for blue mold (P. tabacina) control were conducted at eight different geographical locations. Blue mold failed to develop in three North Carolina tests, although the disease was present in the counties where the tests were located. Blue mold did develop, however, in tests in Puerto Rico and Mexico. Preliminary analysis and comparison of results indicate that various fungicide treatments do not perform the same at different locations. Some of the variable response can be explained by differences in sensitivity of P. tabacina to metaxyl (Ridomil) at the different locations. However, the efficacy at other locations was unexpected. Another result was that oxadixyl performed similarly to metalaxyl at all three test locations, i.e., both were efficacious in Puerto Rico and both showed activity in Mexico. This finding supports the hypothesis that these two chemicals have a common mode of action, and therefore, oxadixyl is not a viable alternative should metalaxyl resistance develop in North Carolina. From the time of the first report of blue mold in North Carolina on July 14 of this year, the disease was monitored and Extension Agents and growers were kept informed on the disease's progress. There was no indication of metalaxyl resistance in the state and the final losses in Burley tobacco due to the disease were negligible.

Contingency plans were made to monitor and test P. tabacina isolates in North Carolina for sensitivity to metalaxyl in the event blue mold developed on metalaxyl treated burley tobacco in 1987. However, no such reports were recorded. Educational information on blue mold was provided to growers in various newsletters, during Burley Day programs, and at burley field tours and field days.

MEXICAN PROJECT

On site visits were made to Mexican tobacco production areas and the NCSU test sites in December 1987 and January 1988. In addition to evaluating the CORESTA collection and breeding line tests, NCSU and R. J. Reynolds personnel had the opportunity to observe first hand the efforts of TABACOS MEXICANOS (TABAMEX) in controlling blue mold. Under the unpredictable, frequent, long-lasting or severe weather conditions (rain, cloudy and cool weather) typical of the Gulf zone, their fungicide evaluation and spray program remains less than adequate, especially in the presence of the metalaxyl resistant strain of P. tabacina. However, 1987-88 was a relatively dry year in the Gulf zone and losses due to blue mold were less than previous years, although the disease was present throughout the state of Veracruz. To better evaluate and compare relative efficacies of fungicides and application strategies, a standard test was designed and conducted at four locations with attention to appropriate checks, application technology and disease assessment. Data from these tests will be submitted in a separate report.

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BIOLOGY AND PATHOGEN VARIATION (With Mary Menetraz and H. W. Spurr, Jr.)

Efforts to develop monogenic host resistance in Europe and Australia have met with limited success because of natural variation in pathogenecity and adaptability within the P. tabacina population. Ecologic (temperature tolerance) and fungicide resistant strains have been documented in North and Central America. A cooperative, in-depth study of pathogen variation was initiated in January, 1987 with the USDA Foreign Disease and Weed Laboratory, Frederick, Maryland. Such information on spore morphology, CORESTA response, isoenzyme/genetic differences, etc. will help us prepare for the risks our growers face. Also, the success of our host resistance and chemical control programs at NCSU will depend upon a better understanding of the variation within the pathogen population.

Eleven isolates of P. tabacina were collected and preserved between 1979-1986 from diverse populations in the southern United States. The potential for genetic variability within the collection was examined in 1986 using starch gel electrophoresis. Sporangia of each isolate were produced and screened for the presence of 32 enzymes of which 20 were positively identified. Similarity of the enzyme bands indicated little variability between the populations. The apparent lack of genetic variability may be due to the absence or failure of sexual recombination within the pathogen population and/or the inability of oospores that are produced to germinate. This could help explain the absence of indigenous metalaxyl resistant strains in Southeast whereas such strains have been observed in tropical overwintering areas where the oospore is thought to be functional. Seventeen enzymes coded as homogygous loci. Heterozygous loci coding for adenylate kinase, isocitrate dehydrogenase, and glucosephosphate isomerase were observed. This indicates that chromosome number of sporangia produced during asexual cycle of P. tabacina is either diploid (2N) or dikaryotic (N+N). Genetic diversity was identified at the allelic level in this study, therefore isozyme analysis could prove to be a valuable tool for assessing pathogenic variability among pathogen populations.

During 1986, we also investigated worldwide pathogenic variability using the CORESTA blue mold trap collection results from 1978-1980. This collection of eight cultivars (differentials) contains the known sources of resistant germplasm to blue mold and was evaluated in 59 separate experiments representing 24 countries in Europe, North and Central America during this nine year period. Multivariate analysis of this large data set indicated ecologic and pathogenic variability probably exists and which can be partitioned into at least seven temporal and/or spatial patterns (clusters) representing the world distribution of P. tabacina. Field tests conducted in North and Central America fell into only two of the seven potential pathogenecity/response clusters.

To investigate this variability further, we are now making a world collection of P. tabacina. Live isolates from 30 countries are being collected and transported to containment facilities at Frederick, MD where they are being stored for further study. The necessary quarantine permits

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have been obtained and collection/shipping protocols have been formulated. To date, exotic isolates have been collected on the east coast of Mexico in February, 1987 where metalaxyl resistance and an apparent variation in systemic blue mold exists. Isolates have also been received from Poland, France, Lebanon, Germany, Costa Rica and Honduras. This effort will continue in 1988-89.

Oospores of two distinct morphological types have been observed in the diseased tobacco tissues. Samples of diseased tissue are being collected, fixed and examined for oospores from as many US and other world locations as possible. This should give further evidence of where and to what extent genetic recombination might be occurring.

HOST SPECIFICITY AND SPORULATION STUDIES (Mary Menetrez and H. W. Spurr, Jr.)

A series of biochemical interactions between the tobacco leaf and fungal spores appear necessary to the development of a functional relationship between host and pathogen. Our first objective is to identify the biochemical components responsible for the specific interaction of P. tabacina with tobacco leading to colonization by the fungus. The ability of spores to adhere to the surface of a host is a key step in the initiation of disease. P. tabacina spores were placed on several host and non-host surfaces and found to adhere to all of them. Treating the spores with a protease resulted in a significant decease only in binding to tobacco leaf surfaces. It appears therefore, that adhesion is a general property of P. tabacina, but the mechanism of adherence to tobacco via a protein may be specific. Interactions of leaf surface chemicals with leaf surface microorganisms may determine the ability of some pathogens to cause disease. Evaluations were made of tobacco leaf surface chemicals on germination of spores of P. tabacina. Sucrose esters, monols and a hydrocarbon fraction were not inhibitory. Cis-abienol at 50ppm inhibited germination by 50%. The duvatrienediols (diols) at the initial concentration of 50ppm completely inhibited germination. When the cis-abienol fraction or the diols were diluted to concentrations found naturally on the tobacco leaf, spore germination was stimulated.

Membrane bound receptors and their complimentary signals have been implicated in cell/cell recognition. Such a mechanism could determine specificity of P. tabacina for Nicotiana. A lectin, a cell wall or cell mebrane protein that binds carbohydrates may be invovled. A crude extract of tobacco leaf protein was tested for lectins and the results were negative. Demonstrating lectin activity by salt concentration, pH of solution, cofactors such as Mg++ or Ca++ and temperature. Tests which precisely control all these variables will be initiated to determine the existence of a tobacco or fungal lectin.

Several environmental factors influence induction of sporulation of P. tabacina. However, with all factors being ideal, if atmospheric humidity is not at or near 100% saturation sporulation does not occur. Our second

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objective is to determine how high humidity induces sporulation of P. tabacina in infected leaves. The precise temperature, photoperiod and atmospheric humidity for sporulation to occur in our system has been determined. We began non-destructive monitoring of plants during disease development and found that leaf surface pH did not change significantly. We also began examining the influence of environment on the disease process. Continuous light did not have an effect on spore germination, but plants kept in the light during the sporulation process sporulated poorly. Light appears to alter the effect of humidity on sporulation. The effects of environment on the plant and the fungus. Sampling the whole plant will not detect subtle, local physiological changes. We plan to monitor the plant microenvironment immediately surrounding the fungus.

EPIDEMIOLOGY AND BIOMETEROROLOGY (With J. M. Davis)

Biometeorological studies have focused on long-distance, airborne movement of inoculum (asexual fungus spores) necessary for new disease outbreaks each year and continued spread of the epidemic. A computer-assisted mapping technique, i.e. meteorological trajectory analysis, is being developed at NCSU that permits the tracking and documentation of probable inoculum pathways and identification of source regions. The SE United States, SW Texas and Mexico are all known or suspected sources of inoculum for North Carolina.

Plant pathologists confirmed the 1985 mid-June occurrences of primary blue mold lesions in six south-central Kentucky counties which are aligned in a north-south direction from Monroe County on the Tennessee border to Mercer County in central Kentucky. Infected Nicotiana repanda plants located in south-central Texas were hypothesized to be the inoculum source. On-site inspection in Texas confirmed moderate to heavy sporulation on these plants from 10 May to 30 May. Atmospheric trajectory analysis supports the south-central Texas source region. Evidence supplied by trajectory analysis, weather radar composite charts, and the alignment of county first occurrence dates supports a late May-early June arrival date for the inoculum and the importance of wet deposition processes in bringing the spores to the leaf surfaces.

Precipitation scavenging can result from in-cloud or below-cloud processes. The latter mechanism appears to be the important one in the Kentucky case. Trajectory analysis, radar mosaics, and the spatial pattern of the first occurrence reports for blue mold all seem to indicate that the arrival of the spores from south-central Texas coincided with the occurrence of showers and thunderstorms. These storms are very efficient below-cloud scavengers of spores. Thus, there is a high probability that precipitation scavenging was the key meteorological event in the initiation of the disease.

Several new transport models have been evaluated. The Branching Atmospheric Trajectory (BAT) model provides a much needed refinement in modeling the diurnal changes in the structure of the Atmospheric Boundary Layer (ABL), within which inoculum is both released and transported.

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During the daytime, the ABL is a well-mixed layer 1 to 2 km thick. Spores released into this layer are soon mixed throughout. At night a surface-based inversion is formed, and the efficient vertical exchange processes characteristic of the daytime ABL are absent. The noctural ABL is often marked by dramatic changes in horizontal wind speed and direction with height. Thus spores located within the ground-based inversion are likely to be transported in a different direction and at a different speed than those above the inversion. This model is now fully operational at NCSU and is being used to examine both historic and new transport case studies providing a more realistic picture of the diurnal variations occurring in the lower atmosphere. Recently, the MESOPUFF II model has been made operational which incorporates an enhanced meteorological data base. This allows for greater spatial and temporal resolution of the lower atmosphere where the spores actually travel. A study of blue mold risk climatology analysis was conducted to integrate, in a probabilistic sense, the spatial and temporal fields of temperature, moisture and general atmospheric features in order to identify geographic areas at high risk to blue mold. This is a relatively new thrust and a long term effort.

The blue mold pathogen, P. tabacina, is thought to overwinter in SC Texas thereby providing inoculum in the spring and early summer as the N. repanda populations develop naturally from seed. A pilot study was initiated, under a Memorandum-of-Agreement with Southwest Texas State University, to more accurately assess the strength of this inoculum source and the characteristics of the indigenous blue mold fungus in Texas. During 1987, working with a botanist from Texas who was familiar with the area and the habitat of N. repanda, we surveyed 16 of the 65 counties composing south-central Texas. As luck would have it, 1987 was a very poor year for N. repanda; warm winter temperatures and very abnormally high rainfall promoted the growth of grass and other plants which competed severely with N. repanda populations. Few N. repanda were found, even at sites where we had observed large populations in previous years. This stratified, random survey of some five million acres, is being repeated in 1988 and the number of sampling sites (counties) is being doubled to increase the probability of finding blue mold infected N. repanda. Using such data, it is hoped to make a reasonable estimate of inoculum source strength (infected plants) which will become a necessary input into future trajectory analyses and possibly forecasting programs. Concurrently with the Texas survey, NCSU/SEPEL Phytotron studies are being conducted to determine the sporulation capacity (time and maximum numbers of spores) on N. repanda and N. tabacum under ideal environmental conditions using various plant growth stages. We are attempting to simulate the age structure of N. repanda populations (communities) and their relative, extended inoculum production periods, i.e., source strength and duration. This information is also necessary to support future forecasting models.

INTERNATIONAL SYMPOSIUM

The Departments of Plant Pathology and Crop Science sponsored an "International Symposium on the Blue Mold of Tobacco" at the Brownestone Hotel, Raleigh, from February 14-17, 1988. Advance publicity and flyers

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were distributed in June, 1987 to more than one thousand individuals, state and federal agencies, universities, and industry companies in the U.S. and foreign countries. Eighty-five persons registered and the attendance ranged from 80-100 both days. Those participating came from 17 countries.

The purpose of the symosium was to assemble, at one meeting, scientists and other interested parties from around the world who have experience with blue mold research and the problems resulting from blue mold epidemics. Sessions were organized on Biology and Epidemiology, Physiology and Biochemistry, and Disease Control. Sixteen major research papers and seven shorter research communications were presented during the two day event. Ample time was scheduled for free and open discussion following each session. Those in attendance indicated they gained a great deal of information and insight into the blue mold problem. They especially appreciated the discussions. We feel that the symposium exceeded our expectations and that the original objectives were achieved. The participants prepared chapters of their contributions which will be reviewed, printed and published as a symposium proceedings by December 1, 1988.

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ACKNOWLEDGEMENTS

The blue mold epidemic is a complex biological phenomenon that can result in serious economic impact on North Carolina growers and the tobacco industry. History has proven this to be the case. The blue mold program at North Carolina State University is committed to finding practical solutions that should lessen the risk our farmers face each year. At the same time, basic research on the nature of the host-pathogen-environment components of the epidemic are being investigated. The blue mold-tobacco system represents one of the most promising case studies for phytopathological research available today. The challenges are many and the opportunities are exciting. The expertise and efforts of some nine scientists are involved in the research described in this report.

J. M. Davis	Meteorology and Plant Pathology
Mary Menetrez	Post-doctorate, USDA, Oxford
N. T. Powell	Extension/Research, Plant Pathology, Flue-cured
Rebeca Rufty	Crop Science
T. J. Sheets	Pesticide Residue Research Laboratory
P. B. Shoemaker	Extension/Research, Plant Pathology, Burley
H. W. Spurr, Jr.	USDA Oxford, Plant Pathology
E. A. Wernsman	Crop Science
C. E. Main	Coordinator, Plant Pathology

The interest and support of the R. J. Reynolds Company is gratefully acknowledged, particularly the input of Richard Reich, Arnold Mitchem, Brian Smeaton, Jerry Shiffert and D. Layton Davis.

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VII. Papers Prese

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Title: N. C. 05307 Disease Control Strategies for Burley Tobacco and Vegetables in Western North Carolina

Project Leader: P. B. Shoemaker

I. Summary of Research:

A. Resistance and soil fumigation for tobacco black root rot (BRR) (with D. Shew). Two tests to evaluate resistance in currently available cultivars and two tests to evaluate various soil fumigants for control of black root rot (Thielaviopsis basicola) on burley tobacco were conducted in grower's fields. Soil assays to determine T. basicola populations were taken before treatment application, after treatment and at the end of the season. Data from these and 1986 test results will provide the basis for making grower recommendations. Results to date indicate that growers with fields with a history of black root rot could benefit by use of either a resistant cultivar or by soil fumigation. However, based on a recent survey of the prevalence of black root rot in burley fields conducted by graduate student, Julie Meyer, it appears that a relatively low percentage (ca. 15%) of fields surveyed have populations of T. basicola high enough to justify control measures.

B. Alternative fungicides and strategies for blue mold (with C. E. Main). Fungicide tests for blue mold (Peronospora tabacina) control were conducted at eight different geographical locations. Blue mold failed to develop in three North Carolina tests, although the disease was present in the counties where the tests were located. Blue mold did develop, however, in tests in Puerto Rico and Mexico. Preliminary analysis and comparison of results indicate that various fungicide treatments do not perform the same at different locations. Some of the variable response can be explained by differences in sensitivity of P. tabacina to metalaxyl (Ridomil) at the different locations. However, the efficacy of fosetyl-Al (Aliette) at one Mexico site and lack of efficacy at other locations was unexpected. Another result was that oxadixyl performed similarly to metalaxyl at all three test locations, i.e., both were efficacious in Puerto Rico and both lacked activity in Mexico. This finding supports the hypothesis that these two chemicals have a common mode of action, and therefore, oxadixyl is not a viable alternative should metalaxyl resistance develop in North Carolina. From the time of the first report of blue mold in North Carolina on June 14, the disease was monitored and Extension Agents and growers were kept informed of the disease's progress. There was no indication of metalaxyl resistance in the state and final losses due to the disease were negligible.

VII. Papers Presented at Professional Meetings:

Shoemaker, P. B. and Main C. E. 1987. Fungicides for blue mold. Presented at Tobacco Workers' Conference, Baltimore, MD, 12-15 Jan.

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Source: https://www.industrydocuments.ucsf.edu/docs/psyk0000

Shew, H. D., Shoemaker, P. B. and Rufty, R. C. 1987. Studies on black root rot on burley in North Carolina. Presented at Tobacco Workers' Conference, Baltimore, MD, 12-15 Jan.

Shoemaker, P. B. 1987. Fungicides, application and strategies for blue mold control. Invited paper for Cuarto Seminario de Intercambio de Conocimientos Sobre el Tabaco, Oaxaco, Oax, Mexico, 6-9 Sep.

IX. Acknowledgements:

The cooperation of the staffs at the Mountain and Upper Mountain Research Stations, TABAMEX, and burley extension agents in W.N.C. who assisted in this work, and the financial support by R. J. Reynolds is gratefully acknowledged.

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Title: NC05638 Modelling Interactions Between Plants and Pests

Project Leader: S. M. Schneider

I. Summary of Research:

The focus of this project is the development of crop production system models. A project of this size must be broken down into smaller parts which are later re-assembled into the larger whole. Crop production models can be divided into three broad component areas; 1) the plant model, 2) appropriate pest models, and 3) models of the dynamic interaction between the plant and associated pests. Work in these areas is described below.

Tobacco Growth Model - Tobacco cvs. NC2326 (susceptible to black shank, Granville wilt, rootknot nematode, and tobacco mosaic virus) and K-399 (highly resistant to black shank, Granville wilt, and rootknot nematode) were planted in 45 plots containing 22 plants each at the Oxford Research Station in 1986 and 1987. Each plot had a guard row on either side of it and two guard plants at each end. One plot of each variety was randomly chosen to be sampled each week from transplanting to final harvest. Measurements included leaf length, width, fresh weight, dry weight, surface area, and concentration of sugars, starches, alkaloids, and nitrogen. Air and soil temperature, rainfall/irrigation, and solar radiation were monitored continuously. This large data set is being used to characterize plant growth and development for inclusion in a computer simulator of the tobacco plant. Quantified relationships include rate of leaf initiation, rate of leaf expansion for each stalk position, and rate of increase of chemical constituents at each stalk position. These relationships are a function of environmental conditions, time, and plant growth stage. The plots will be continued for a third year. Results will be compared for the period 1986-88. The data set will be used to test the tobacco growth simulator.

Interaction between rootknot nematode and tobacco cultivars. Three rootknot species, Meloidogyne incognita (Mi), Meloidogyne arenaria (Ma), and Meloidogyne javanica (Mj) were inoculated onto tobacco cvs. NC2326 (susceptible) and K-399 (resistant to Mi). Plants were sampled 1, 4, 7, and 14 days after inoculation. Numbers of Mi and Ma steadily increased in roots of NC2326, but numbers of Ma were much lower than Mi. Numbers of Mi in K-399 were initially higher than in NC2326, but decreased rapidly after 4 days. Numbers of Ma were similar in NC2326 and K-399 up to 7 days, then increased in NC2326 and decreased in K399. Results with Mj were inconsistent. These data suggest that the nematode readily penetrates the resistant cultivar, but leaves the root when a feeding site cannot be established. Some level of resistance to Ma may also be present.

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Tobacco cvs NC2326, K-399, and Speight G-28 were inoculated with Mi and Ma. Three days after inoculation, roots were gently rinsed, and re-planted into nematode-free soil. On NC2326, both Mi and Ma readily developed to mature females within 21 days. Fewer Mi and no Ma reached the adult stage on K-399. Neither Mi nor Ma reached the adult stage on Speight G-28. These are preliminary results. Further tests are underway.

Interaction of soil moisture and Meloidogyne incognita on tobacco. Tobacco cv NC2326 was transplanted into microplots and inoculated with one of six levels of Mi or Ma. Plots were treated with a high or low water irrigation treatment. Tobacco growth measurements were made throughout the season. Yield and quality were determined at harvest. There was a significant interaction between water treatment and initial inoculum level (Pi), but the effect of Pi alone was not significant.

Results of a microplot experiment testing the influence of soil type and rootknot nematode on tobacco growth were confounded by a mixing of resistant and susceptible seed before planting in the seed bed. Field plots were inoculated with rootknot nematode and planted to a susceptible host to build up the nematode population for next year's field experiments. (Cooperators: K. R. Barker and T. Wheeler.)

Modelling soybean cyst nematode (SCN) and its interaction with the soybean plant. A project has been initiated to model SCN, identify the linkages between SCN and the host plant, quantify the linkages, write a computer simulator of SCN, and interface it to SOYGRO, a soybean simulator. The literature has been reviewed and the structure of the SCN model chosen. Some of the plant processes which may be influenced by SCN are root nodulation, water uptake, root respiration, and carbohydrate partitioning. SCN processes which may be impacted by changes in host condition are development rate, mortality, sex ratio, and reproduction. Data gaps have been identified and experiments planned. (Cooperators: K. R. Barker, D. P. Schmitt, and S. Pao-sheng.)

II. Graduate Student:

Terry Wheeler, Ph.D. student in plant pathology. Thesis topic: Interaction of soil moisture and Meloidogyne incognita on tobacco. (co-advisor).

IV. Publications:

Schneider, S. M. 1987. Stage-specific population development and fecundity of Paratrichodous minor. J. of Nematology 19: 395-403.

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VI. Manuscripts in Review:

Schneider, S. M. Root-knot nematodes on tobacco. In Compendium of Tobacco Diseases (H. D. Shew and G. B. Lucas, eds).

Schneider, S. M. Problems associated with life cycle studies of soil-inhabiting organisms. In Lecture Notes in Statistics (L. MacDonald and B. Manly, eds).

VII. Papers presented at Professional Meetings:

Schneider, S. M. Penetration of tobacco roots by Meloidogyne incognita, Meloidogyne arenaria, and Meloidogyne javanica. Ann. Mtg. Society of Nematologist, Honolulu, HI, July 1987.

Schneider, S. M. Problems associated with life cycle studies of soil-inhabiting organisms. Symposium on Estimation and Analysis of Insect Populations sponsored by the U. S. National Science Foundation - New Zealand Cooperative Science Program and the University of Wyoming, Laramie, WY. Jan. 1988.

VIII. Acknowledgements:

The technical assistance of Marion Tuck and A. P. Currin, Jr. is gratefully acknowledged.

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Title: NC 3901 Plantbed and field management of flue-cured tobacco.

Project Leader: G. S. Miner

I. Summary of Research:

Studies were continued to further evaluate the effectiveness of Basamid as an alternative to methyl bromide for fumigation of plantbeds. Weed control, transplant production and transplant quality were similar for methyl bromide and Basamid applied under plastic in the fall. Fall-applied Basamid without plastic also gave similar levels of transplant production but weed control was slightly less effective. Spring-applied Basamid was less suitable than when applied in the fall because of wet soil conditions and residual toxicity effects on tobacco transplants. Assuring that plantbeds are well-aerated at seeding time is more risky for spring-applied compared to fall-applied Basamid.

The effects of premature flowering on yield and chemical composition of flue-cured tobacco were evaluated at three locations. A preliminary threshold level of 15-20% premature flowering has been established below which no management input is required before normal topping. However, topping and cleaning up of early flowering plants is more labor intensive than it is with normal plants. Yield is reduced markedly at higher levels of premature flowering.

Methods of sampling flue-cured tobacco were compared and the results were presented at the annual review meeting of experiment station supervisors and tobacco staff. People who contributed to this project were Dr. Long, Dr. Weeks, Darryl Dunnigan and Tom Bartholomew. The results are presented at the end of the report for the record.

II. Graduate Students: Two Ph.D. candidates

IV. Publications:

1. Miner, G.S., A.Z. Galindez, and M.R. Tucker. Response of flue-cured tobacco to foliar and soil application of Mn. Tob. Sci. 31:28-31.

2. Smith, W.D., G.F. Peedin, W.K. Collins, M.R. Tucker, G.S. Miner, and E.J. Kamprath. Tobacco response to sulfur on soils differing in depth to the argillic horizon. Tob. Sci. 31:36-39.

3. Vepraskas, M.J. and G.S. Miner. Tillage effects on alkaloid production in tobacco as influenced by soil texture, root abundance, and rainfall. Tob. Sci. 31:52-56.

4. Vepraskas, M.J., G.S. Miner, and G.F. Peedin. Relationships of soil properties and rainfall to effects of subsoiling on tobacco yield. Agron. J. 79:141-146.

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A Comparison of Sampling Methods for
Chemical Analyses of Cured Tobacco

Introduction

Summarization of on-farm and research station tobacco experiments usually includes an evaluation of the effects of treatment variables on the chemical composition of cured leaves. Inferences are made on the effects these treatments will have on the suitability of the tobacco for smoking purposes. Often, more emphasis is placed on this aspect of tobacco evaluation than on agronomic parameters such as yield.

Collection of representative samples of cured leaves, whose analyses accurately reflect the organic and inorganic chemical composition of harvested material, is an extremely important component of the extension and research tobacco programs in North Carolina. Since tobacco is harvested in three to five leaf groups and the weights among leaf groups are highly variable, a sample that is representative of the total harvested leaf must reflect the differential weight contributions of each leaf group to the whole plot sample. Thus, the proportional weight contribution of each leaf group to the whole sample is taken into account and a composite sample (composited by weight) is collected. Failure to do this accurately or inclusion of errors in weighing the individual leaf groups can greatly affect the analytical results obtained.

To illustrate the relationship between a mean reducing sugar concentration, a composite-mean reducing sugar concentration, and the effect weighing errors can have on the composite-mean reducing sugar concentration, a hypothetical data set is given in Table 1.

Table 1. Leaf weight and concentration of reducing sugars in leaves harvested in four primings.*

Stalk Position		Leaf Weight	Reducing Sugars
		lb/A	%
A		300	8.0
B		600	13.0
C		900	18.0
D		1200	20.0
	Total	3000	

*Assume a 40g sample is collected in each case.

A. Simple mean sugar concentration:

With 4 primings, we could take 10g of each stalk position and our analysis would reflect a simple mean sugar concentration.

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(We can divide by 4 below because contributions from each stalk position are equal: 10g).

$$\text{Sugar} = \frac{\Sigma \text{ \% sugar in all stalk positions}}{4} = \frac{59}{4} = \underline{14.8\%}$$

Intuitively, we know this value is not representative of the whole plant because the upper stalk positions contribute much more to the total harvest weight than the lower stalk positions.

B. Composite - Mean sugar concentration:

With 4 primings, we make up our composite sample with proportionate contributions from each stalk position. Thus, for a 40g sample, the weights of each are given below:

$$\text{A: } \frac{300}{3000} \times 40\text{g} = 4\text{g}$$

$$\text{B: } \frac{600}{3000} \times 40\text{g} = 8\text{g}$$

$$\text{C: } \frac{900}{3000} \times 40\text{g} = 12\text{g}$$

$$\text{D: } \frac{1200}{3000} \times 40\text{g} = 16\text{g}$$

$$\text{Sugar} = \frac{\Sigma \text{ weight x \% sugar in each priming}}{\text{total weight of sample taken}}$$

$$\text{Sugar} = \frac{\Sigma(4\text{gx}8\%)+(8\text{gx}13\%)+(12\text{gx}18\%)+(16\text{gx}20\%)}{40\text{g}}$$

$$\text{Sugar} = \frac{672\text{g}\cdot\%}{40\text{g}} = \underline{16.8\%}$$

This value is representative of the whole plant because individual weight contributions of each priming are accounted for.

C. Effect of errors in weighing primings for composite sample.
If we make a mistake and weigh out 14g of priming D instead of 16g, the composite-mean value is low (still divide by 40g because that is presumed sample size).

$$\text{Sugar} = \frac{\Sigma(4\text{gx}8\%)+(8\text{gx}13\%)+(12\text{gx}18\%)+(14\text{gx}20\%)}{40\text{g}}$$

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20%)

$$\text{Sugar} = \frac{632\text{g}\cdot\%}{40\text{g}} = 15.8\%$$

For an error where 18g of D is weighed out (2g over), the same calculations showing a value of 17.8% will result. Thus, small weighing errors can greatly affect the composite-mean values obtained, especially, if they occur in primings contributing the most weight to the sample.

Since sampling can greatly affect the analyses of cured leaf, a comparison of methods of sampling was undertaken with the following objectives:

1. Evaluate the effect of sample size on variability in analytical results,
2. Evaluate the effect of random versus nonrandom leaf selection on variability in analytical results, and
3. To compare whole versus half-leaf lamina selection on variability in analytical results.

Materials and Methods

Samples were collected from four treatments of a rate of N by rate of harvest experiment at the Upper Piedmont Research Station. Four replications of each treatment were sampled for a total of 16 plots. The intent was to obtain tobacco with a range in alkaloid and sugar concentrations.

The sampling methods chosen for comparison are listed in Table 2:

Table 2. Sampling methods used in comparative study.

Sample Weight	Leaf Composite by Weight		Priming[a]	
	Nonrandom-Whole	Random-Half	Core	Random-Half
g				
30	x	x	x	x[b]
50	x	x		
100	x			

[a] The priming sample weights were variable but approximated 30g. Since each priming was analyzed separately and composite mean values were calculated based on priming weights, the actual weight is of no importance.

[b] Hereafter referred to as priming method.

All methods were sampled in duplicate to obtain a measure of sampling variability. The first sample of nonrandom whole-leaves were obtained from leaves taken on a weighted basis from the top of the priming bundles. Then the bundles were turned over and the second sample was taken. This was

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expected to give the greatest variability in duplicate samples. The random half-leaves were pulled from throughout the priming bundle for duplicate samples for both the composite samples and where samples were collected by priming. Two-inch diameter core samples were then taken through each priming bundle near the center and the duplicate was taken in the same manner about one to two inches from the site of the first core sample.

All samples were ground, thoroughly mixed, and analyzed for total alkaloids and reducing sugars. Analytical results for the core and priming samples were combined on a weighted basis to provide whole plant data which could be compared to composite-sample data. The experimental treatments (N x harvest rate) were evaluated by analysis of variance for each sampling method and coefficients of variance (C.V.) and sampling errors (between duplicates) were used to compare sampling methods.

Results and Discussion

The analyses for total alkaloids and reducing sugars for the 16 plots by all methods are shown in Tables 3 and 4, respectively. In attempting to understand the results, a summation of differences between duplicate samples in each plot is given at the end of each table for each sampling method. The larger the sum of differences, the greater the variation between duplicate samples. Thus, the core method was least repeatable by duplication for total alkaloids (Table 3). The 30 and 50g, nonrandom whole-leaf methods and the 50g, random half-leaf method also had rather large summation differences whereas the remaining three methods were similar.

Although summation of differences between duplicate samplings is indicative of the repeatability of sampling, it can be affected greatly by a large difference between duplicates in one plot. Statistics associated with an analysis of variance of field treatments (N rate x harvest rate) by each sampling method are better indicators of sampling performance. The two statistics presented are coefficient of variation (C.V.) and sampling error. For total alkaloids, the magnitude of the C.V.'s generally followed the magnitude of summation differences among sampling methods (Table 3). The sampling errors associated with the core and 30 and 50g, nonrandom whole leaf methods further substantiate that these sampling methods are less repeatable than the other methods.

Assuming the best possible job was done in mixing the ground sample, then the results obtained by mathematically calculating a weighted mean (whole-plant) value from the priming data represents the best theoretical results that can be obtained. Mean alkaloid values were highest for the priming method and were underestimataed by the core method. Alkaloids are known to be concentrated near leaf margins so sampling cores through the center of priming bundles results in a nonrepresentative sample for alkaloids and is unacceptable. Alkaloid levels for all nonrandom whole-leaf methods were lower than those for the random half-leaf methods, and the latter were most comparable to the priming method.

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Table 3. Comparison of analyses for total alkaloids by seven sampling methods.

Plot	Duplicate Sample	Composite: Nonrandom Whole Leaf 30g	Composite: Nonrandom Whole Leaf 50g	Composite: Nonrandom Whole Leaf 100g	Composite: Random Half Leaf 30g	Composite: Random Half Leaf 50g	Priming: Core	Priming: Random-half
2	1	2.97	2.97	2.93	3.15	3.02	2.04	3.04
	2	2.93	2.91	2.98	3.10	3.32	2.35	2.93
Difference (D)		0.04	0.06	0.05	0.05	0.30	0.31	0.11
3	1	2.39	2.61	2.76	3.07	2.82	1.89	2.79
	2	3.03	2.72	2.40	2.67	2.69	2.05	2.65
	D	0.36	0.11	0.36	0.40	0.13	0.16	0.14
4	1	2.24	2.10	2.32	2.64	2.39	1.76	3.02
	2	2.55	2.07	2.09	2.58	1.88	1.77	2.33
	D	0.31	0.03	0.23	0.06	0.51	0.01	0.69
7	1	3.13	3.13	2.77	3.09	3.08	2.01	3.00
	2	3.14	3.05	2.83	2.85	3.37	2.34	3.26
	D	0.01	0.08	0.06	0.24	0.29	0.33	0.26
10	1	2.28	2.33	2.62	2.55	2.44	1.66	2.96
	2	2.58	2.17	2.24	2.49	2.29	1.99	3.36
	D	0.30	0.16	0.38	0.06	0.15	0.33	0.40
13	1	3.02	2.92	2.94	3.09	2.97	2.02	2.91
	2	2.93	3.15	2.97	3.08	3.05	2.20	2.96
	D	0.09	0.23	0.03	0.01	0.08	0.18	0.05
15	1	2.60	2.06	2.41	2.28	2.57	1.84	2.55
	2	2.37	2.92	2.55	2.54	2.54	2.05	2.62
	D	0.23	0.86	0.14	0.26	0.03	0.21	0.07
18	1	2.68	2.66	2.80	2.57	2.77	1.63	2.76
	2	2.63	2.42	2.87	2.61	2.67	2.02	2.68
	D	0.05	0.24	0.07	0.04	0.10	0.39	0.08
19	1	2.32	2.26	2.37	2.44	2.38	1.79	2.21
	2	2.02	2.19	2.27	2.55	2.36	1.65	2.28
	D	0.30	0.07	0.10	0.11	0.02	0.14	0.07
22	1	2.53	2.43	2.71	2.96	3.10	1.82	3.04
	2	2.67	2.43	2.53	2.63	2.57	2.23	2.87
	D	0.14	0.00	0.18	0.33	0.53	0.41	0.17
24	1	2.86	2.83	2.96	3.05	2.92	1.78	2.73
	2	3.15	2.80	3.09	2.89	2.97	2.25	2.87
	D	0.29	0.03	0.13	0.16	0.05	0.47	0.14
26	1	2.01	2.13	2.04	2.10	2.30	1.69	2.28
	2	2.18	1.99	2.12	2.05	2.05	1.75	2.29
	D	0.17	0.14	0.08	0.05	0.25	0.06	0.01
28	1	2.82	2.45	2.78	2.84	2.73	2.17	2.82
	2	2.36	2.39	2.77	2.56	2.86	2.41	2.84
	D	0.46	0.06	0.01	0.28	0.13	0.24	0.02
34	1	2.40	2.52	2.65	2.49	2.39	1.73	2.55
	2	3.07	2.57	2.50	2.38	2.51	1.85	2.50
	D	0.33	0.05	0.15	0.11	0.12	0.12	0.05
35	1	2.23	2.21	2.32	2.52	2.60	1.92	3.08
	2	2.34	2.39	2.41	2.58	2.51	1.71	3.04
	D	0.11	0.18	0.09	0.06	0.09	0.21	0.04
36	1	2.86	3.25	2.80	3.16	3.17	1.72	3.04
	2	3.28	2.78	3.07	2.95	2.99	2.24	3.19
	D	0.42	0.47	0.27	0.21	0.18	0.52	0.15
Σdifferences		3.61	2.77	2.33	2.43	2.96	4.09	2.45
C.V.(%)		8.80	7.52	4.76	4.99	6.25	10.61	5.76
Means		2.64	2.56	2.62	2.70	2.70	1.95	2.80
Sampling Error		0.054	0.037	0.016	0.018	0.028	0.043	0.026

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Table 4. Comoarison of analyses for reducing sugars by seven methods on 16 plots.

Plot	Duplicate Sample	Composite: Nonrandom Whole Leaf 30g	Composite: Nonrandom Whole Leaf 50g	Composite: Nonrandom Whole Leaf 100g	Composite: Random Half Leaf 30g	Composite: Random Half Leaf 50g	Priming: Core	Priming: Random-half
		%						
2	1	15.8	16.0	15.7	14.2	14.3	16.6	16.8
	2	14.1	16.5	15.5	15.4	16.6	17.1	16.8
Difference (D)		1.7	0.5	0.2	1.2	2.3	0.5	0.0
3	1	17.6	17.8	17.4	17.6	17.0	18.0	19.3
	2	18.5	16.2	19.8	18.7	18.8	16.5	19.7
	D	0.9	1.6	2.4	1.1	1.8	1.5	0.4
4	1	18.2	18.8	19.2	13.4	17.9	16.4	19.1
	2	16.6	20.8	18.0	18.0	19.6	17.0	17.9
	D	1.6	2.0	1.2	4.6	1.7	0.6	1.2
7	1	14.6	13.8	13.9	14.5	15.0	15.4	16.3
	2	12.2	11.4	12.3	15.5	13.2	15.3	14.4
	D	2.4	2.4	1.6	1.0	1.8	0.1	1.9
10	1	16.5	18.2	17.1	20.1	18.5	17.5	20.5
	2	21.1	19.1	19.4	19.0	19.0	17.8	19.9
	D	4.6	0.9	2.3	1.1	0.5	0.3	0.6
13	1	12.6	14.2	11.0	14.9	15.4	15.3	16.7
	2	14.6	13.5	14.2	13.4	14.7	15.3	17.1
	D	2.0	0.7	3.2	1.5	0.7	0.0	0.4
15	1	11.1	15.0	14.6	14.1	14.9	14.3	14.5
	2	15.0	12.8	13.5	13.2	14.1	14.3	14.9
	D	3.9	2.2	1.1	0.9	0.8	0.0	0.4
18	1	16.3	15.9	16.1	18.1	18.0	17.3	18.1
	2	17.1	17.0	14.4	17.3	17.8	17.8	17.3
	D	0.8	1.1	1.7	0.8	0.2	0.5	0.8
19	1	18.0	16.5	13.2	16.0	16.2	15.9	16.7
	2	16.8	15.9	15.4	15.9	16.2	15.4	15.7
	D	1.2	0.6	2.2	0.1	0.0	0.5	1.0
22	1	15.6	17.0	14.9	15.5	14.9	15.8	16.4
	2	15.3	14.2	16.1	16.7	15.5	16.0	15.6
	D	0.3	2.8	1.2	1.2	0.6	0.2	0.8
24	1	13.1	14.6	12.4	13.2	12.3	12.3	14.6
	2	11.9	13.0	12.3	13.1	11.9	12.9	13.7
	D	1.2	1.6	0.1	0.1	0.4	0.6	0.9
26	1	19.9	18.7	19.1	18.2	18.7	16.7	19.6
	2	19.0	18.4	19.7	18.7	18.6	17.5	19.1
	D	0.9	0.3	0.6	0.5	0.1	0.8	0.5
28	1	16.7	15.9	16.7	15.9	14.6	17.0	16.0
	2	19.6	19.9	14.2	15.1	13.8	17.0	15.3
	D	2.9	4.0	2.5	0.8	0.8	0.0	0.7
34	1	20.7	20.9	17.6	19.8	19.6	17.6	20.3
	2	19.1	19.8	18.4	20.9	18.0	17.2	19.0
	D	1.6	1.1	0.8	1.1	1.6	0.4	1.3
35	1	18.7	19.8	18.0	16.8	17.9	17.4	19.0
	2	20.9	19.3	19.4	17.5	19.5	18.2	19.2
	D	2.2	0.5	1.4	0.7	1.6	0.8	0.2
36	1	17.0	16.1	15.1	16.2	16.3	15.3	17.8
	2	15.2	17.0	15.4	16.2	18.2	15.8	16.9
	D	1.8	0.9	0.3	0.0	1.9	0.5	0.9
Σ differences		30.0	23.2	22.8	16.7	16.8	7.3	12.0
C.V.(%)		9.3	7.4	7.4	6.3	5.5	2.7	3.5
Means		16.5	16.7	15.9	16.3	16.5	16.2	17.3
Sampling Error		2.371	1.533	1.407	1.055	0.812	0.191	0.371

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ng
on-half

16.8
16.8
0.0

19.3
19.7
0.4

19.1
17.9
1.2

16.3
14.4
1.9

20.5
19.9
0.6

16.7
17.1
0.4

14.5
14.9
0.4

18.1
17.3
0.8

16.7
15.7
1.0

16.4
15.6
0.8

14.6
13.7
0.9

19.6
19.1
0.5

16.0
15.3
0.7

20.3
19.0
1.3

19.0
19.2
0.2

17.8
16.9
0.9

12.0
3.5
17.3
0.371

The summation of differences between duplicates, C.V.'s, and sampling errors indicate the core method was the best for reducing sugars while the priming method was almost as good. The reason for the superiority of the core method is that sugars are uniformally distributed throughout leaf lamina and the duplicate cores were taken adjacent to each other. The random half-leaf composite sampling methods were almost as good as the priming method but nonrandom half-leaf composite methods were highly variable. Mean reducing sugar levels were similar among all methods although slightly higher for the priming method.

An analysis of variance combined over all sampling methods was completed with field treatments as whole plots and sampling methods as subplots in a split-plot arrangement (Table 5). Differences in main effects of field treatments and methods of sampling were significant for both total alkaloids and reducing sugar concentrations in cured leaves. The significant main effect means are shown in Table 6. Both sugar and alkaloid levels were significantly higher for the priming method compared to the other methods. The reason is not apparent. In general, there were no significant differences in sugars or alkaloids among the nonrandom whole-leaf and random half-leaf methods but the core method was inferior to the other methods for alkaloids.

Since alkaloid levels for the core sampling method were considerably below those obtained by other sampling methods, a linear regression model was used in an attempt to predict alkaloid levels using values obtained by the core method. The relationship between alkaloid levels by the priming method and the core method is:

$$\hat{Y} = 1.09 + 0.875\ X \quad \text{with } R^2 = 0.31$$

where X = alkaloid levels for coring. Although there is a relationship between the two parameters, variability is such that predicted alkaloid levels would not be very reliable. Thus, this method of compensating for low alkaloid levels by the core method is totally unacceptable.

Conclusions

1. Although suitable for reducing sugars, the core method is unacceptable for obtaining cured leaf samples for analysis of total alkaloids.

2. Although there was less variability in analyses of total alkaloids and reducing sugars among all methods, with the exception of the core method, than was expected, those with nonrandom selection of whole leaves tended to be less repeatable than those with random selection of half-leaves. Moreover, levels of total alkaloids and reducing sugars tended to be lower.

3. Random selection of half-leaves in either a 30g or 50g sample gave results similar to the priming method. Because these sample sizes fit well with present grinding procedures and delivery (return) of analytical results,

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Table 5. Combined analysis of variance for reducing sugars and total alkaloids across treatments and methods of sampling.

Variance Source	Degrees of Freedom	Sum of Squares	F Test	Probability of a > F
		Reducing Sugars		
Replication (R)	3	104.7		
Treatment (T)	3	583.0	15.7	0.0006
Error A	9	111.1		
Sample (RxT)	16	18.8		NS
Method (M)	6	35.1	2.7	0.0484
RxM	18	39.2		
TxM	18	34.4		NS
RxTxM	54	79.7		
Residual Error	96	105.0		
		1111.0		
Total	223			
		Total Alkaloids		
Replication (R)	3	1.84		
Treatment (T)	3	8.84	6.8	0.0110
Error A	9	3.92		
Sample (RxT)	16	0.44		NS
Method (M)	6	15.36	107.3	0.0001
RxM	18	0.43		
TxM	18	1.28		NS
RxTxM	54	2.27		
Residual Error	96	3.12		
Total	223	37.50		

Table 6. Mai

Field I.D.	
Low N-Late Ha	
Low N-Early H	
High N-Late H	
High N-Early	
High N-Late H	
High N-Early	
Low N-Late Ha	
Low N-Early H	

a/ WL = whole

b/ Values wit different

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total

Probability of a > F

0.0006

NS
0.0484

NS

0.0110

NS
0.0001

NS

Table 6. Main effect means ranked according to Duncan's Multiple Range Test.

Field Treatment		Sampling Method	
I.D.	Mean	I.D.[a/]	Mean[b/]
		Reducing Sugars (%)	
Low N-Late Harvest	18.2 a	Comp.-Priming	17.3 a
Low N-Early Harvest	17.9 a	50g,WL,NR	16.7 b
High N-Late Harvest	15.6 b	30g,WL,NR	16.5 b
High N-Early Harvest	14.3 c	50g,HL,R	16.5 bc
		30g,HL,R	16.3 bc
		Comp.-Core	16.2 bc
		100g,WL,NR	15.9 c
		Total Alkaloids (%)	
High N-Late Harvest	2.82 a	Comp.-Priming	2.80 a
High N-Early Harvest	2.69 b	30g,HL,R	2.70 b
Low N-Late Harvest	2.42 c	50g,HL,R	2.69 b
Low N-Early Harvest	2.33 d	30g,WL,NR	2.64 bc
		100g,WL,NR	2.62 bc
		50g,WL,NR	2.56 c
		Comp.-core	1.95 d

[a/] WL = whole leaf; HL = half leaf; NR = nonrandom; R = random.

[b/] Values within columns not followed by the same letter are significantly different at the 5% level of probability by Duncan's Multiple Range Test.

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this method (i.e., 30- or 50-gram, random half-leaf) should probably continue to serve as the basis for taking samples for chemical analyses.

4. The greatest source of error may be associated with weighing the correct amount of leaves from each priming for composite samples. Small weighing errors on upper stalk primings can greatly affect analyses when presumed total sample weights are used as illustrated under Table 2. Thus, insuring that accurate scales of the proper type are available, that they are tared properly, and that workers take the time to weigh the proper amount of lamina from randomly selected half-leaves is crucial to this phase of tobacco evaluation.

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Title: NC03659 Environmental Determinants of Crop Growth and Soil Productivity

Project Leader: C. D. Raper, Jr.

I. Summary of Research:

A. Use of hydrostatic pressure as a method of collecting xylem fluid (with J. K. Vessey).

Xylem sap frequently is sampled and analyzed in physiological studies to investigate aspects of root metabolism and ion transport. However, excision of the shoot and collection of the fluid that exudes from the stem due to root pressure, which is the common method of sampling xylem sap, may not accurately represent the *in situ* composition of xylem sap. Since hydrostatic pressure can be adjusted to produce a flow of xylem sap comparable to that which occurs in an intact, actively transpiring plant, the *in situ* composition may be more accurately reflected in xylem fluid produced from cut stems by application of hydrostatic pressure to root systems which are enclosed in "pressure bombs."

Application of 100 kPa of hydrostatic pressure to excised roots of soybean plants enclosed in a pressure bomb resulted in a sustained flow of xylem fluid from the stem for up to 6 hours at a rate approximately equivalent to the calculated rate of xylem flow in an intact, actively transpiring plant. Analysis of fractions of xylem fluid collected over time indicated that a steady-state concentration of cations and anions occurred during only the first 1.0 to 1.5 hours, after which the concentrations declined. In tobacco, however, a steady-state rate of xylem exudation was not obtained with application of hydrostatic pressure, and ion concentrations in the xylem fluid begin to decline immediately. These results indicate that, while application of hydrostatic pressure may be useful in accurately sapling xylem fluid from excised root systems of some species for limited periods, the method has limited application for investigations of ion transport from roots to shoots of tobacco.

B. Interaction between elevated concentrations of atmospheric CO_2 and nitrogen supply (with L. Tolley-Henry and J. K. Vessey).

While elevated atmospheric carbon dioxide generally enhances growth of crop plants, the magnitude of response is dependent upon other environmental factors. Numerous investigations have indicated that plants grown with elevated atmospheric carbon dioxide have a greater requirement for nutrients, and particularly nitrogen, to support the increase in dry matter production. Most of these studies, however, have been conducted using a solid substrate as a culture medium. Although

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photosynthesis may increase with elevated carbon dioxide, accompanying decreases in stomatal conductance generally reduce transpiration. Decreased transpiration effectively decreases movement of solution through the substrate to the root system. As a result, mass flow of nitrate to replenish nitrate depletion by plant uptake at the soil-root interface is reduced, and unless nitrate fertilization is increased, decreased transpiration can lead to decreased acquisition of nitrogen from a solid medium. In hydroponic culture, however, availability of nitrate is not dependent on mass flow. This suggests that the effect of elevated carbon dioxide on nitrogen acquisition and utilization from a solid medium may be an indirect effect on nitrogen availability rather than a direct effect on nutritional requirement of the plant. This hypothesis was evaluated by growing plants under atmospheric concentrations of carbon dioxide of 400, 650, and 900 µL/L in flowing hydroponic culture at solution concentrations of 0.5, 1.0, 5.0, and 10.0 mM nitrate.

II.

Accumulation of neither dry matter nor nitrogen by plants was not affected during vegetative growth by nitrate concentration in solution over the range of atmospheric concentrations of carbon dioxide. Furthermore, the effect of elevated concentrations of carbon dioxide on dry matter and nitrogen accumulation by plants was not significant. Thus, plant response to elevated levels of carbon dioxide is not directly limited by changes in plant requirement for nitrogen and to capacity of the roots to absorb nitrate.

III.

IV.

C. Effect of pH on nitrogen uptake from mixed nitrate and ammonium sources (with S. Chaillou, J. K. Vessey, and L. Tolley-Henry).

V.

In previous experiments we have shown that at a solution pH of 6.0 tobacco, as well as soybean and tomato, can utilize ammonium as the sole source of nitrogen as effectively as nitrate as the sole source. As pH is decreased, however, uptake rates per gram root dry weight decrease when nitrogen is supplied as ammonium and increase when nitrogen is supplied as nitrate. Thus, when nitrogen is supplied from mixed nitrate and ammonium sources, the relative efficiency of uptake and utilization of each ion may be influenced by pH of solution. To investigate the effects of pH on "preferential" uptake of nitrate or ammonium from mixed sources, plants are being grown in hydroponic culture containing 1.0 mM NO_3^-, 0.67 mM NO_3^- plus 0.33 mM NH_4^+, 0.33 mM NO_3^- plus 0.67 mM NH_4^+, or 1.0 mM NH_4^+ and over a pH range of 4.5 to 6.5.

VI.

Experiments have been completed at pH 6.0 and 5.5. At pH 6.0, dry matter and total nitrogen accumulation was equal when either nitrate or ammonium was available as the sole source of nitrogen. When both nitrate and ammonium were supplied together at either ratio, uptake of ammonium was equal to the uptake of ammonium as the sole source and uptake of nitrate was half the uptake of nitrate as the sole source. Although total accumulation

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of nitrogen from the mixed sources was 50% greater than from either sole nitrogen source, the increase in total nitrogen uptake was not accompanied by an increase in dry matter production. Presumably, the nitrogen absorbed as ammonium was assimilated and utilized to support growth and the nitrogen absorbed as nitrate was stored as nitrate. Plant material is being analyzed to confirm the partitioning of nitrogen.

Plants exposed to mixed sources of nitrate and ammonium at pH 5.5 absorbed less ammonium and more nitrate than plants at pH 6.0. Furthermore, plants supplied with ammonium as the sole source of nitrogen accumulated less total nitrogen than plants supplied with nitrate only or a mixture of nitrate and ammonium. As the investigation is continued with experiments at pH 5.0 and 4.5, a greater dependence of the plant on absorption of nitrogen from nitrate is expected.

II. Graduate Students:

Tom C. Granato (Ph.D.)
James W. Rideout (Ph.D.)

III. Postdoctoral Fellows:

Dr. Leslie Tolley-Henry
Dr. J. Kevin Vessey

IV. Publications: None

V. Manuscripts Accepted for Publication:

Tolley-Henry, L., C. D. Raper, Jr., and T. C. Granato. 1988. Cyclic variations in nitrogen uptake rate in soybean plants: Effects of external nitrate concentration. J. Expt. Bot. 39:in press.

Raper, C. D., Jr., J. F. Thomas, L. Tolley-Henry, and J. W. Rideout. 1988. Assessment of an apparent relationship between availability of soluble carbohydrates and reduced nitrogen during floral initiation in tobacco. Bot. Gaz. 149:in press.

VI. Manuscripts in Review:

Granato, T. C., and C. D. Raper, Jr. Proliferation of maize (Zea mays L.) roots in response to localized supply of nitrate. J. Expt. Bot.

Lim, J. T., C. D. Raper, Jr., H. J. Gold, and G. G. Wilkerson. Incorporation of measured photosynthetic rates in a mathematical model for calculation of nonstructural carbohydrate concentration. J. Expt. Bot.

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Lim, J. T., G. G. Wilkerson, C. D. Raper, Jr., and H. J. Gold. A dynamic model of vegetative soya bean plants. I. Model structure and behaviour under varying root temperature and nitrogen concentration. J. Expt. Bot.

Lim, J. T., H. J. Gold, G. G. Wilkerson, and C. D. Raper, Jr. A dynamic model of vegetative soya bean plants. II. Sensitivity analysis. J. Expt. Bot.

VII. Papers Presented at Professional Meetings:

Granato, T. C., and C. D. Raper, Jr. 1987. Effects of exogenous nitrate supply on the rate of respiration, reduced N concentration, growth, and morphology of corn roots. Annual Meeting, Crop Science Society of America, Atlanta, Georgia, 4 December 1987.

Raper, C. D., Jr. 1987. Measurement and control of ionic composition using automated ion chromatography. Annual Meeting, American Society of Horticultural Science, Orlando, Florida, 9 November 1987.

VIII. Graduate Student Theses Completed:

Granato, T. C. 1987. Effects of exogenous nitrate on processes that produce proliferation of corn roots. Ph.D. Thesis.

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Title: NC03827 Cultural, Nutritional and Varietal Investigations with Burley Tobacco

Project Leader: J. E. Shelton

I. Summary of Research:

This project has the following objectives:

A. To evaluate methods of distinguishing differences in P and K requirements of soils with differing chemical and physical characteristics.

B. To correlate information from objective 1 with yields and quality of burley over a wide range of soil P and K levels and soil management systems.

Although soils of western North Carolina used for burley tobacco production may vary considerably in their physical and chemical characteristics and respond differently to fertilize amendments all soils having a given soil test level for P and K would receive the same recommendations.

In 1987 soils having cation exchange capacities (CEC) ranging from 4.5 to 10.0 me/100g treated with potassium to give a calculated % K saturation of 3, 5, or 7% resulted in saturation percentage of 2.5-9.5%. On a soil having a CEC of 4.5 the cured leaf yields were 2184, 2352 and 2800 kg/ha at K saturation percentages of 3.5, 5.7, and 9.5% respectively. Topdressed K applied 30 days after setting increased yields at the 3.5 and 5.7 % K saturations but not at the 9.5% K level. With the low rainfall of 1987 possible leaching of K may have been minimal with preplant applications supplying adequate K for maximum growth. Two locations having CEC's of 6.5 and 7.0 me/100g resulted in cured leaf yields of 3360 of 3584 kg/ha at K saturations of 8.4 and 8.5%, respectively, but had apparently not reached maximum yields.

Soils having CEC's of 7.8 and 7.9 me/100g gave maximum yields of 3248 and 3472 kg/ha at 7.9 and 7.0% K saturations, respectively. Three sites having CEC's greater than 8.0 me/100g gave maximum yields at 5.2 to 5.7% K saturations.

The results of these investigations indicate that a high % K saturation would be required on low CEC soils and that such soils may not retain adequate K for maximum yields from a single preplant application.

A high P fixing soil with a high soil test P level receiving rates of 0, 84, 165, or 252 kg P_2O_5/ha in 1984 gave significant yield increases to annual applications of 60 kg P_2O_5/ha regardless of the initial rate of application.

IX. Acknowledgements

Appreciation is expressed to Mr. Dwayne Tate for excellent technical assistance and to county extension agents, Wiley DuVall and Jerry Washington, for help in selecting cooperating growers and assistance in conducting on-farm research projects.

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Title: NC 3998 THE EFFECT OF TILLAGE AND COVER SPECIES ON NITROGEN MOVEMENT IN MOUNTAIN AGRICULTURAL SYSTEMS.

Project Leader: G. D. Hoyt

I. Summary of Research

This project has the following objectives:

A. To establish conservation tillage for burley tobacco production in the mountain region on soils subject to erosion.

B. To determine optimum fertilizer source and placement for conservation tilled burley tobacco.

C. To determine the effect of various tillage systems on tobacco growth, yield and root development.

Various management systems were established in 1987 to further evaluate the consequences of conservation tillage and cover crop residues on tobacco production. Previous research concluded that rye or barley residue utilized with a conservation tillage program could provide competitive yields with conventional culture and provide needed reduction in erosion. In our third year of comparing conventional, strip-till and no-till tobacco yield results conclude that conventional tillage yields greater (approximately 200 kg/ha) than the two forms of conservation-tillage (only in 1987 were they statistically different). Transplant survival was greater for conservation-tilled (95%) than for conventional tillage (80% survival). A strip-till tobacco experiment was established to compare fertilizer application techniques for improving tobacco growth early in the growing season. Both broadcast vs. banded and surface vs. incorporated cultural practices were compared with little differences found among the treatments. Banded-incorporated fertilizer treatment did increase yield >250 kg/ha but was not significantly different than the other three treatments. The third experiment involved the use of three winter covers (bare, vetch, and rye) with 4 N rates. This experiment will be a strip-till tobacco/no-till corn experiment on a two-year rotation. First year tobacco results show that tobacco grown on rye cover treatment flowered earlier and continued to have greater percent flowering throughout the growing season than either the bare ground or vetch cover treatments. The highest N rates for the bare ground and rye cover treatment produced a higher percentage of tobacco flowering among N rates, whereas vetch cover treatments showed lower flowering percentage at high N rates early in the season, with greater flowering percentage when plants were counted late in the season. Bare and vetch cover treatments produced similar and greater tobacco yields than rye cover treatments (mean N rate). Tobacco yields also increased with N addition, with lowest yields from rye covers with 0 and 75 kg N/ha rate, and no difference among covers at the 225 kg N/ha rate.

IV. Publications

Hoyt, G. D. 1988. Conservation tillage for burley tobacco: Soil Fertilizer Movement, pp. 25-28. In: 1988 Burley Tobacco Information. N. C. Agricultural Extension Publication. AG 376.

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Hoyt, G. D. 1987. Legumes as a green manure for conservation tillage. pp. 96-98. In: J. F. Power (ed). The Role of Legumes in Conservation Tillage Systems. Soil Conservation Society of America. Ankeny, Iowa.

Gilliam, J. W. and G. D. Hoyt. 1987. Effect of conservation tillage on fate and transport of nitrogen. pp. 217-240. In: T. J. Logan, J. M. Davidson, and M. R. Overcash (eds). Effects of Conservation Tillage on Ground Water Quality. Lewis Publishers, Inc., Chelsea, Michigan.

Hoyt, G. D. and A. D. Cole. 1987. Soil nitrogen movement and transformation in conservation tilled tobacco. Soil Sci. Soc. North Carolina Proc. 30:3-10.

Hoyt, G. D. 1987. Conservation tillage for burley tobacco, pp. 22-24. In: 1987 Burley Tobacco Information. North Carolina Agricultural Extension Publication. AG 376.

V. Manuscripts accepted

Vepraskas, M. J. and G. D. Hoyt. 1988. Comparison of the Trench-Profile and Core Methods for Evaluating Root Distributions in Tillage Studies. Agron. J. 80: In press.

VII. Papers presented at professional meetings

"Legumes as a green manure in conservation tillage." G. D. Hoyt. Presented before The Role of Legumes in Conservation Tillage Systems national conference. Athens, Georgia, April, 1987.

XI. Acknowledgements

I wish to thank Mr. Anthony Cole for his excellent technical assistance on this project.

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TOBACCO LITERATURE SERVICE

Annual Report for the Calendar Year 1987

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		1986	1987	Change
I.	TOBACCO ABSTRACTS.			
	Abstracts published	1,978	2,026	+ 48
	Publications searched	6,100	6,050	— 50
	Average per month	508	504	— 4
	Distribution			
	Domestic:			
	Exchange	49	49	Nc
	Complimentary	109	103	— 6
	Subscriptions	97	101	+ 4
	Total	255	253	— 2
	Foreign:			
	Exchange	141	143	+ 2
	Complimentary	27	25	— 2
	Subscriptions	137	140	+ 3
	Total	305	308	+ 3
	Total, All Categories	560	561	+ 1
	Copies Per Year *	2,800	3,927	+1127
II.	TOBACCO REPRINT SERIES.			
	Reprints published (#722-734)	8	12	+ 4
	Recipients	165	164	— 1
	Copies distributed	1,320	1,968	+ 648
III.	BIBLIOGRAPHIC INFORMATION SERVICE			
	Requests served	1,100	1,083	— 17
IV.	PHOTOCOPY SERVICE.			
	Tobacco Staff	18,944p	30,363p	+11,419
	Paid Orders	4,376p	2,867p	— 1,509
V.	OTHER PUBLICATIONS DISTRIBUTED.			
	"Recent Advances in Tobacco Science".	59	104	+ 45

* 7 issues published in calendar year 1987

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ADDENDUM

Listed below, by department, are research activities which received supplemental funding from the North Carolina Tobacco Foundation, Inc. for the period July 1, 1987 through June 30, 1988.

Activity	Investigator(s)
Biological and Agricultural Engineering	
Burley Mechanization	C. W. Suggs
Measurement of the Optical Properties of Tobacco as Related to Texture, Body and Color	W. F. McClure R. E. Williamson F. G. Giesbrecht
Investigation of Alternative Means of Removing Moisture from Tobacco	C. F. Abrams, Jr. S. C. Mohapatra
Application of Expert Systems to Tobacco Production Decisions	R. S. Sowell
Botany	
Metabolic Activity in the Shoot Apex of *Nicotiana tabacum* in Relation to Leaf and Floral Initiation	J. F. Thomas
Crop Science	
Genetic Investigations on the Angular Leaf Spot and Wildfire Dieases of Tobacco Caused by *Pseudomonas syringae* pv. *tabaci*.	R. C. Rufty E. A. Wernsman
Analytical Service of Experimental Flue-Cured Tobacco	W. W. Weeks
Physiology of the Growth and Development of Burley Tobacco	R. C. Long P. E. Barney, Jr.
Development and Application of Haploid Selection Theory for Tobacco Improvement	E. A. Wernsman

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Activity	Investigator(s)
Crop Science (continued)	
Genetic Studies of Resistance to Black Root Rot (*Thielaviopsis basicola*) in Burley Tobacco and Variation in the Pathogen Population in North Carolina	R. C. Rufty
The Use of Activated Characoal to Enhance the Tolerance of Flue-Cured Tobacco to Certain Herbicides	F. H. Yelverton A. D. Worsham G. F. Peedin
Carbon-Nitrogen Interactions in Tobacco Plants Acclimating to Nitrogen Stress	T. W. Rufty, jr. R. J. Volk
Genetic and Breeding Studies for Bacterial Wilt Resistance in Tobacco	G. R. Gwynn
No-Till Tobacco	A. D. Worsham R. L. Davis
Economics and Business	
An Empirical Analysis of Seasonal Labor Costs and the Impact of Recent Changes in Federal Regulations: With Consideration of the Effects on Structural Issues	D. A. Sumner
Entomology	
Effects of Host-Plant Health on the Bionomics of the Green Peach Aphid	E. P. Lampert
Monitoring Insect Abundance, Development of Insect Data Bases and Archiving of Insect Abundance Information	E. P. Lampert
Influence of Crop Rotation and Tillage Practices on the Level of Biological Control of Tobacco Soil Insect Pests	G. J. House D. M. Jackson D. P. Schmitt
Entomology — (Pesticide Residue Research Laboratory)	
Residues of Fenamiphos on Flue-Cured Tobacco	T. J. Sheets R. B. Leidy

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Activity	Investigator(s)
Entomology -- (Pesticide Residue Research Laboratory) (continued)	
Total Residue Method for Metalaxyl on Flue-Cured Tobacco	T. J. Sheets R. B. Leidy
Genetics	
Genetic Modification of Tobacco	D. F. Matzinger
Utilization of Recombinant DNA Technology to Explore Gene Regulation and Genetic Engineering of Tobacco	M. A. Conkling
Microbiology	
Study of Anti-Fungal Interactions of Tobacco Surfaces	L. W. Parks
Plant Pathology	
Epidemiology of Tobacco Black Shank	H. D. Shew
The Influence of Host Resistance or Tolerance in Burley Tobacco on Viral Pathogenesis	J. W. Moyer G. V. Gooding, Jr. R. C. Rufty
Biological Control and Genetic Studies of Granville Wilt in Naturally Infested Tobacco Fields	E. Echandi G. R. Gwynn
Somaclonal Variation for the Development of Disease Resistance in Tobacco	M. E. Daub
Control of Flue-Cured Tobacco Diseases by Means of Cultural Practices, Varietal Resistance, and Chemical Application	N. T. Powell
Black Root Rot on Burley Tobacco, Its Distribution and Importance	P. B. Shoemaker H. D. Shew
Development of a Computer Simulation Model of the Tobacco Production System	S. M. Schneider

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Activity	Investigator(s)
Plant Pathology (continued)	
Genetic Diversity of *Meloidogyne incognita* Populations and Biological Resistance Mechanisms in Tobacco	K. R. Barker N. T. Powell A. C. Triantaphyllou
Field Evaluation of Control Strategies for Tobacco Viruses	G. V. Gooding, Jr. E. P. Lampert
Soil Science	
Mechanisms of Flower Induction in Flue-Cured Tobacco and Management Alternatives for Premature Flowering of Field-Grown Tobacco	G. S. Miner C. D. Raper, Jr.
Tillage Systems for Burley Tobacco Production	G. D. Hoyt
Evaluation of Methods of Determining the Fertilizer Requirements of Varying Soil Types and Promotion of Use of Soil Test in Production of Quality Burley Tobacco	J. E. Shelton
General	
Director's Reserve for Emergencies and Reserve Research Assistantships	W. H. Johnson

Please keep in mind that the information presented in this annual report is NOT FOR PUBLICATION but merely represents preliminary findings which may later appear in published form. We trust that you will find the information presented herein of interest and value.

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